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# The Chemical Nature and Distribution of Black Pigment in Soil

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# THE CHEMICAL NATURE AND DISTRIBUTION

# OF

# BLACK PIGMENT IN SOIL

By

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# THE CHEMICAL NATURE AND DISTRIBUTION OF BLACK PIGMENT IN SOIL

## Introduction

A problem of this type necessarily involves a study of soil humus, a field which has probably been more confused than any other phase of chemistry. The term humus was frequently used during the time of the Romans to indicate the soil as a whole. Later it was applied to the organic matter of soils, or to different fractions of this organic matter. Most of the earlier literature diversely referred to humus as humic acid, ulmic acid, mull acid, et cetera, but since these acids are of very complex structure, and are probably not even definite chemical compounds it served only to confuse the subject. Although the term humus was not always used to designate the same preparations, it was most frequently used to indicate that portion of the soil organic matter which is soluble in dilute alkali solutions. In reviewing the literature it becomes evident that our knowledge of the organic chemistry of soils has developed step by step along with the changing conceptions of soil humus.

Soil organic matter consists of a great mass of substances of plant, animal, and microbiological origin. It embraces organic substances in various stages of decomposition and therefore includes compounds which were present in the original plant, compounds which compose the bodies of microorganisms, materials that have been synthesized by organisms, and all of the great variety of compounds which may be formed from the above sources by decomposition. The latter consisting of processes of hydrolysis, oxidation and reduction, or various other chemical reactions

which may take place in converting dead organic material into living protoplasm or into the end products of carbon dioxide, nitrogen, and water.(16) A certain portion of this heterogeneous mass is more or less resistant to further decomposition and remains for a time in the soil, or even may accumulate in the soil, under certain conditions. This dark brown to black fraction is often known as humus. However, as used in this paper humus refers to that portion of the soil organic matter peptized by four per cent ammonium hydroxide. That fraction peptized by four per cent ammonium hydroxide, precipitated by acids, and insoluble in alcohol, is designated as <u>black pigment</u>.(16) It is called humic acid by many writers.

<u>Theories of formation</u>

Since the time of Liebig chemists have been greatly interested in soil organic matter. The very voluminous literature on the subject is evidence of this. However, even though there has been a vast amount of research directed along this line, and our knowledge about soil organic matter has increased tremendously, final solution as to the exact nature of humus has not been forthcoming. The vagueness concerning the nature of humus, black pigment, or humic acid, has been aided by a lack of knowledge of its origin. Therefore, in order to point out more clearly the nature of the problem, a brief review of the literature dealing only with the phase of humus formation will be given.

Early theories were all agreed that plant substances were sources of humus. De Saussure as an advocate of this hypothesis held that any plant material, when in contact with moist air, became blackened, and this constituted soil humus.

In 1889 Hoppe-Seyler[19] demonstrated that cellulose and hemicellulose would not form humus. Trussov[57] in 1915 verified this and in one paper stated that typical black humus was formed only when lignin, proteins, tannins, and pigments were present. However, he maintained that starches, sugars, celluloses, et cetera, could serve as indirect sources of humus since these could be used as food by the soil microorganisms. He considered the protoplasm of bacteria and fungi as a possible source of humus. From this point of view then, all substances that can be used as food by microorganisms could be considered as indirect sources of humus. This is the first theory that definitely sets forth microorganisms as playing a dominant role in humus formation.

Early researches along this line consisted of extraction of the soil humus, usually by dilute alkalies. Further investigation was then directed at the isolated product but since this complex is insoluble, more or less non-reactive and of colloidal nature, real progress was retarded. The investigators merely obtained fractionations of the material by a limited number of solvents, such as pyridine, dilute alkalies, and alcohol. These fractions, although not of definite composition, were given such names as crenic and apocrenic acids, humin, hymatomelanic acid, sacculmic acid, nitrolin, lignohumic acid, nitrohumin, sucrohumic acid, et cetera, which in reality have no real chemical significance and tend only to confuse the issue.

Schreiner & Shorey[50],[51] in a series of papers reported the isolation of a number of organic compounds of definite composition. Soil humus was shown to consist of a great variety of organic compounds, their chemical nature being as varied as that of the plant or animal

material from which humus originates.

However, these results certainly did not settle the humus problem. All of their researches were carried out on the filtrate after precipitation of the black pigment by various acids. No work was attempted on the less reactive, black, colloidal fraction. Furthermore, the compounds isolated by Schreiner & Shorey make up a very small part of the total humus and might be considered as impurities of the humus, or a few of them may even have been formed by the reagents used in the extraction and detection.

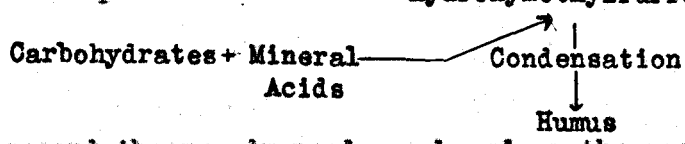
It was only natural that the organic chemist should then turn his attention to the problem of synthesizing humus as a means of determining its chemical nature. Papers dealing with this phase of the work are too numerous to mention, but we can group practically all of the important contributions under four distinct theories of formation.

1. One of the earliest theories advanced was based on the formation of artificial "humic acids" by interaction of carbohydrates with mineral acids. One of the first workers to advance this idea was W. B. Bottomley,(5) who treated sugars with acids and obtained humic acid.

Sugars + HCL $\longrightarrow$ Humic Acid + Humin Substances

Other investigators held that humus was produced by polymerization of furfural, a compound which is produced when pentosans are treated with hot acids. The furfural is then converted by strong acids into a black insoluble mass. Marcusson,(26)(27)(28) a disciple of this hypothesis, concluded that humic acid was an intermediate product between wood and

coal. On the basis of his investigations he assumed that humic acid consisted of polymerized peri-difurans. Beckley[4] assumed the following reaction to take place:




2. A second theory advanced was based on the assumption that humus was formed by the condensation of amino acids or polypeptides with carbohydrates. Many of the early workers held that nitrogen in soil humus was an impurity, but it soon was established that nitrogen was an integral part of the complex. This theory apparently was developed to overcome the fact that no nitrogen was present in synthetic humus prepared from carbohydrates and mineral acids. Maillard[24][25] as an advocate of this hypothesis showed that reducing sugars plus alpha-amino acids formed substances similar to natural humus. Also that glycylglycine reacts with xylose or glucose and forms brown humus-like products. He concluded nitrogen was essential for humification.

3. Still a third theory advanced by various workers explained the origin of humus by the oxidation of aromatic compounds. Eller[10] prepared a substance which was very similar to humus in appearance and in certain reactions, by oxidizing phenol, pyrocatechol, and hydroquinone in alkaline solution.

Since Gortner[17] suggested a possible relationship between the microorganisms of the soil and the black pigment, considerable work has been done along this line. It was found that certain microorganisms do produce dark colored materials, and it was further demonstrated that the black color produced by certain organisms was due to aromatic

compounds, similar to ortho and para dihydroxybenzene. Stoklasa[54] concluded that humus was an accessory product of the respiration of microorganisms. These results seemed to support the concept that humus was formed by oxidation of aromatic compounds.

Investigators in research work involving the chemistry and origin of coal contributed a great deal of evidence pointing towards the close relationship between lignin and humus. Fischer[11] was the first to propose the theory that lignin was the mother substance of humus. Fischer and Schrader,[11] Fuchs,[14] Maschall and Page,[31] Du Toit and Page,[8] and others upheld this hypothesis.

Thus the attention of investigators was directed toward lignin or lignin-like complexes in their pursuit of the humus problem.

4. More recently a theory was advanced by Waksman[59] which set forth the idea that lignin combines with a protein-like substance to form humus. He places a great deal of emphasis upon the importance of microorganisms, for it is due to their activity both in decomposition and in synthesis that protein material is built up, while carbohydrates and ligneous materials are respectively decomposed and altered. According to his theory these materials, lignins, carbohydrates, and proteins are closely related to the formation of humus either directly or indirectly.

The colloidal organic fraction of any soil is one of the most active principles of the soil. Soil humus regulates the nature of the microbial population, acts somewhat as a storehouse for plant nutrients, and even helps to modify the chemical and physical nature of the soil itself. It accounts for most of the combining power of a soil with bases, it buffers the soil solution, influences soil structure, water-holding capacity,

et cetera, and plays an important role in effecting the stability of the inorganic colloidal fraction. It may either increase or decrease the stability of the inorganic colloidal portion, depending upon the nature of the humus. It likewise effects various other phenomena either directly or indirectly.

The value of humus extract as a plant nutrient has been studied by German investigators. Their results published in 1937, show that the addition of humus to sand media stimulates plant growth as much as many commercial fertilizers.

It can readily be seen that a knowledge of the soil black pigment is very essential if we are to understand the origin and development of soils, as well as the processes that control plant growth.

Since very little is definitely known about the chemical nature of this complex and since most of the early research was performed on the humic acid fraction isolated from peats, it was the object of this investigation to isolate the black pigment from prairie soils, forest soils, and muck soils, and to study each pigment separately. Thus several perplexing questions might be answered. For example, are the black pigments isolated from these three different soil groups similar in their chemical and physical properties? What are some of the physical and chemical properties of the soil black pigment? Does the pigment behave as a true acid? What conclusions can be drawn as to its chemical constitution and method of formation?

Furthermore, these physical and chemical characteristics will be used as a check on future extractions by different peptizing agents to determine whether or not exactly the same fraction or compound has been

isolated in each case.

Likewise it was the purpose of this investigation to study the distribution of this black pigment throughout the great plains area and to correlate it with climate and certain soil groups of the world.

Finally, we wished to ascertain whether or not the black pigment content of a soil could be appreciably increased in the laboratory by addition of different organic materials.

## EXPERIMENTAL

### Extraction of the "black pigments."

Approximately 3 kilograms of a muck soil from Minnesota were thoroughly leached with one per cent hydrochloric acid to remove all traces of soluble calcium. The muck was then transferred to an 18-liter bottle and the humus extracted by four per cent ammonium hydroxide. Various methods of flocculating the clay portion of the suspension were tried but the following method was adopted.

After the muck had been subjected to extraction by intermittent shaking with four per cent ammonium hydroxide for 24 hours, the suspension was allowed to settle for two or three days. In this time the solution had separated into two quite distinct layers. The jet black liquid was siphoned off the top, and passed through a Sharples centrifuge, the cylinder of which had been lined with a tight-fitting sheet of celluloid. Practically all of the clay remaining in suspension was thrown out on the celluloid sheet and discarded.

Four per cent ammonium hydroxide was again added to the muck which remained in the bottle and the extraction was repeated.

The centrifuged suspension was acidified with HCl and the jet black gelatinous precipitate allowed to settle. The pigment could not be filtered even by suction so the clear supernatant liquid was siphoned off and the remaining black colloidal suspension was passed through the Sharples centrifuge. The black pigment was thus thrown out on the celluloid sheet, where it was readily removed. The pigment was dried at 70°C, pulverized in a glass mortar, copiously washed with water, leached with ninety-five per cent ethanol, and again washed thoroughly with water. It was then air-dried and labeled P2. It contained 7.77 per cent ash on an oven-dry basis.

The acid filtrate, after precipitation and removal of the pigment, was usually pale yellow. In many cases when this was made alkaline by adding sodium hydroxide the solution took on a greenish tint. When acidified again the yellow color reappeared, although it was less intense than before.

Extraction of a black pigment having an ash content of only 3.60 per cent on an oven-dry basis, from the gray-brown forest soils of Michigan was accomplished merely by leaching several kilograms of soil with a solution consisting of 4 per cent ammonium hydroxide and 2 per cent ammonium carbonate. The soils having been previously leached with one per cent hydrochloric acid to the absence of calcium in the leachate. The resultant black solution was concentrated, and then acidified with HCl which caused the precipitation of the black flocculent pigment. The suspension was transferred to a carboy and allowed to settle for two days. The supernatant liquid was then siphoned off and water was added, while thoroughly agitating the mixture. This was again allowed to settle for several days and the process was repeated. The pigment was washed eight to twelve times in this manner. Finally, the pigment was transferred to a large evaporating dish and dried under the radiant heater. The temperature never exceeded 70°C. When first extracted the pigment was very voluminous and gel-like. Upon drying it shrank enormously, cracked, and became very brittle. The pigment was ground, washed again with water, leached with 95 per cent ethanol and finally leached with more water. It was then air-dried and labeled P3. Ash = 3.60 per cent on an oven dry basis.

The pigment from the grassland soils of the western great plains

area was extracted in exactly the same manner as that used on the gray-brown forest soils. All of the ammoniacal extracts from the various samples in the plains region used in the study of pigment distribution were collected and concentrated. The concentrate was then acidified and the same procedure as above was used. The pigment from these soils was labeled P1 and had an ash content of 6.86 per cent on an oven dry basis.

PURIFICATION

Various methods of purification were tried with little success. A portion of the dried pigment was redissolved in 4 per cent ammonium hydroxide, the suspension was then acidified and the pigment separated. This procedure was repeated several times but no appreciable decrease in per cent ash was noted.

Another portion of the washed and dried pigment was dissolved in a relatively small amount of ammonium hydroxide and placed in an electrodialysis cell. The suspension was electrodialyzed for 12 hours using 90 volts and 50 amperes. At the end of the 12 hours the solution was neutral to litmus. Most of the pigment was coagulated upon the parchment next to the anode, indicating that the pigment is a negatively charged colloid. The ash content was reduced only very slightly. A portion of the black pigment, P2, was purified by reprecipitation and dialysis to such an extent that its ash content was reduced to 1.20 per cent. But in order to have a sufficient quantity with which to work, all of the fractions were thrown together and the composite used in subsequent analysis.

FRACTIONATION OF PIGMENT

Various attempts were made to fractionate these black pigments by

means of numerous solvents. It was hoped that some crystalline materials might be separated or some fraction obtained that might possess a few definite physical and chemical properties. Such solvents as carbon tetrachloride, petroleum ether, diethyl ether, isopropyl ether, ethyl acetate, chloroform, benzene, acetic acid, oxalic acid, acetone, methyl alcohol, ethyl alcohol, and pyridine were used. However, no such fraction was obtained. In every instance where a fractionation was possible the portion separated was amorphous and colloidal in nature.

ANALYSIS

The nitrogen content was determined by the Kjeldahl method while per cent carbon, and hydrogen was determined by dry combustion. The ash content of each pigment was determined by igniting in an electric furnace at 700-750°C.

| | Per cent C | Per cent H | Per cent N | Per cent O | Per cent Ash |
|---|---|---|---|---|---|
| P1 | 58.35 | 4.54 | 5.88 | 31.2 | 6.86 |
| P2 | 58.81 | 5.54 | 5.49 | 30.2 | 7.77 |
| P3 | 55.58 | 5.17 | 6.03 | 33.2 | 3.60 |

ACETYLATION

Eight grams of the pigment were suspended in 40 cc of acetic anhydride and 10 drops of concentrated sulfuric acid were added. The suspension was then heated in a water bath at 100°C for 8 to 10 hours and was stirred by intermittent shaking. In every case the pigments were insoluble in the anhydride even at 100°C. The contents of the flask were then emptied into a large volume of ice water, thoroughly mixed, and filtered. The acetylated pigment was washed until the filtrate was neutral to

bromthymol-blue. It was next air-dried, ground, and ash and moisture determined. In one instance dry hydrochloric acid gas was introduced into the flask in place of the sulfuric acid. Subsequent analysis showed no difference in acetyl content, between this product and that obtained by using sulfuric acid.

Pigments -1-, -2-, and -3-, were acetylated quite readily even in the cold. Upon the addition of acetic anhydride and sulfuric acid to P1 the contents and flask became warm, with P2 the flask became quite hot, and with P3 the mixture became warm. The following analysis shows the relative amount of acetylation that took place.

| Original Sample | Per cent acetic acid | | Per cent acetic acid |
|---|---|---|---|
| P1 | 1.33 | Ac1 (cold) | 1.76 |
| P2 | 1.00 | Ac2 " | 5.62 |
| P3 | 1.50 | Ac3 " | 2.48 |

The acetylated products were just as insoluble as the original pigments, being peptized only by dilute alkalies.

DETERMINATION OF ACETYL CONTENT

The determination of acetyl content was made on both the acetylated pigments and the original materials. The method employed, with slight modification, was that of Perkin.(43) 0.5 to 1.0 gram of sample along with 25 cc of 95 per cent aldehyde-free ethanol were placed in a round bottom flask, and connected to a condenser. Five cc of concentrated sulfuric acid were added dropwise and the mixture refluxed for 15 minutes. The water in the condenser was then drained and the ethyl acetate was swept out of the reaction flask into an erlenmeyer containing 25 cc of 0.2N alcoholic potassium hydroxide, by a slow stream of alcohol vapor.

This procedure was continued until the volume of the distillate in the receiving flask was approximately 150 cc. During the reaction, which required about one hour for completion, the water bath around the reaction flask was heated at such a rate that the contents of the flask were reduced in volume about one half. The distillate was then refluxed for thirty minutes, diluted with distilled water and the unused potassium hydroxide determined by titration with N/10 hydrochloric acid.

The results are presented in the following table where they are expressed as per cent of acetic acid, and also as milliequivalents of acetic acid per 100 grams of water-free, ash-free sample.

TABLE I

| Sample | Source | Acetyl content | |
|---|---|---|---|
| | | Per cent acetic acid | Milliequivalents of acetic acid per 100 grams of pigment |
| P1 | Variety of grassland soils from the great plains | 1.33 | 22 |
| P2 | Minnesota muck soil | 1.00 | 16 |
| P3 | Michigan forest soils | 1.50 | 25 |
| Ac1 | Acetylation product of P1 | 12.36 | 206 |
| Ac2 | " " P2 | 18.79 | 313 |
| Ac3 | " " P3 | 18.53 | 308 |
| Lignin 15 (*) | Marshall clay loam soil | 1.5 | 25 |
| Ac 15 (*) | Acetylated lignin 15 | 22.9 | 381 |
| Lignin 6 (*) | Corn cobs | 0.2 | 4 |
| Ac 6 (*) | Acetylated lignin 6 | 13.6 | 227 |
| Lignin (x) (√) | Corn cobs | 24.13 | 404 |
| Ac.Lignin (√) | Oat hulls | 23.44 | 392 |

(*) Data from reference (61) (√) Data from reference (44)

The acetyl content of all three pigments is relatively low, and their behavior towards acetylation is very similar to that of lignin isolated from soil and from corn cobs.

## METHYLATION

Pigments -1-, -2-, and -3- were methylated by suspending a 5 gram sample in 100 cc of water containing 10 cc of a 50 per cent solution of potassium hydroxide. The mechanical stirrer was started and 20 cc of dimethyl sulfate was added dropwise. From 10 to 20 cc more of potassium hydroxide was needed in each case to keep the suspension alkaline during the course of the reaction. The mixture became hot as the reaction proceeded. Stirring was continued from 1 to 2 hours at room temperature. The flask was then gradually warmed to 60°C in a water bath and held at this temperature for two hours. The suspension was allowed to stand over night and next morning was poured into a large volume of cold water containing a few drops of sulfuric acid. The mixture was thoroughly agitated and then filtered. The black gelatinous precipitate was dried at 60°C, ground, and thoroughly washed with water. The sample was then returned to the methylating flask and the above procedure repeated. Each sample was methylated three times, or until the methoxyl content became constant. The final product was then filtered, dried and ground. The dry sample was washed with distilled water, 50 per cent alcohol, and finally leached again with copious amounts of water.

The appearance of the three methylated pigments and their behavior towards all solvents were exactly the same as those of the corresponding pigments yet subsequent analysis showed that methylation had taken place.

## DETERMINATION OF METHOXYL CONTENT

The method employed was that of Phillips.[46] Approximately 0.3 gram of sample and 10 cc of hydriodic acid were placed in the apparatus and the flask heated to 130-140°C. The condenser was maintained at a temperature of 50-60°C. A slow stream of carbon dioxide carried the methyl

iodide out of the reaction flask and into the alcoholic silver nitrate. The silver iodide was filtered, washed, dried, and weighed.

The results, calculated as per cent methoxyl and also as millequivalents of methoxyl per 100 grams of sample, on the ash-free, moisture-free basis, are presented in the following table:

TABLE 2

| Sample | Source | Methoxyl content | |
|---|---|---|---|
| | | Per cent Methoxyl | Milliequivalents per 100 grams of sample |
| P1 | Grassland soils of the great plains | 1.03 | 33 |
| P2 | Minnesota muck soil | 1.67 | 54 |
| P3 | Michigan forest soils | 1.74 | 56 |
| Me 1 | Methylated P1 | 8.71 | 281 |
| Me 2 | " P2 | 8.98 | 289 |
| Me 3 | " P3 | 7.92 | 255 |
| Lignin 6 (*) | Corn cobs | 12.9 | 444 |
| Lignin 25(*) | Marshall clay loam soil | 1.6 | 56 |
| Me Lignin 6(*) | Corn cobs | 27.1 | 935 |
| " " 25(*) | Soil | 17.5 | 603 |
| Lignin ) | NaOH extracted | 15. | 484 |
| Me Lignin)(V) | " " | 31.7 | 1022 |

(*) Data from reference (61) (V) Data from reference (45)

## BASE EXCHANGE CAPACITY

Chemical absorption by soil materials has been known since 1850 when it was discussed by Thompson. Way advanced our knowledge on this subject and among other things found that the active absorbent agent was contained in the clay. Since that time a vast amount of research has been directed along this line and the chemistry of base exchange of the inorganic portion of the soil is fairly well understood. However, our knowledge of the base

exchange reaction of organic materials is not so complete.

Muller[38] found that various natural organic materials possessed base exchange properties, and that decomposition by natural processes increased the base exchange capacity of straw and manure. He concluded, that since such drastic measures were used to extract the various organic fractions from the soil, the property of base exchange may be attributed to certain groups such as the hydroxyl and carboxyl, rather than specific chemical substances.

McGeorge[34] showed that base exchange reactions occur in plant materials. Furthermore he illustrated that the exchange capacity of lignin in soils is not consistent but varies in different soils.[33] His results from ten different soils varied from 38 to 178 M.E.per 100 grams, but his aqueous-alkali-soluble ligno-humate showed a much more constant as well as a higher base exchange capacity, e. g. 321 M.E. to 431 M.E. per 100 grams. He also concluded that the lignin content of organic matter is a linear function of the base exchange capacity and that the exchange capacity of the organic material increases as the organic matter passes through successive stages of decomposition in the soil. According to McGeorge[32] lignin, ligno-hemicellulose, and ligno-cellulose or related bodies function largely as the exchange compounds of soil organic matter. He is of the opinion that the exchange capacity in lignin and ligno-humate is a function of phenolic hydroxyl groups.

Millar, Smith and Brown[36] likewise found that the exchange capacity of several organic materials increased as biological decomposition proceeded.

Since the base exchange capacity of organic materials has been

ascribed to phenolic hydroxyl groups and carboxyl groups, the effect of blocking these by acetylation and methylation can perhaps best be studied by means of the base exchange reaction.

Therefore the base exchange capacity of all three pigments, and their corresponding acetylated and methylated products was determined by the following procedure:

A weighed amount of sample, 0.5 to 1.0 gram was placed in a Gooch crucible, leached with 150 cc of neutral, normal calcium acetate solution and then washed with carbon dioxide-free water to the absence of calcium in the filtrate. The sample was then leached with 150-175 cc of 0.1N hydrochloric acid and the calcium content of the filtrate was determined.

After leaching the sample with acid it was washed with distilled water and the procedure was then repeated, using neutral, normal barium acetate solution. The results were calculated in terms of milliequivalents of exchangeable cation per 100 grams of water-free, ash-free material, and are presented in Table 3.

Table 3

| Sample | Source | Base Exchange Capacity M.E. per 100 grams of sample | |
|---|---|---|---|
| | | Calcium | Barium |
| Pigment -1- | Grassland soils from the great plains | 392 | 396 |
| Pigment -2- | Minnesota muck soil | 256 | 292 |
| Pigment -3- | Michigan forest soils | 267 | 239 |
| Ligno-humate (√) | (Average of 10 soils) | 382 | |
| Lignin 6 (*) | Corn cobs | 34 | 21 |
| Lignin 25 (*) | Marshall clay loam soil | 336 | 341 |
| Ac -1- | Acetylated pigment -1- | 345 | 339 |
| Ac -2- | Acetylated pigment -2- | 250 | 245 |
| Ac -3- | Acetylated pigment -3- | 225 | 223 |
| Ac Lignin 6 (*) | Corn cobs | 6 | 11 |
| Ac Lignin 25 (*) | Marshall clay loam | 60 | 87 |
| Me -1- | Methylated pigment -1- | 271 | 275 |
| Me -2- | Methylated pigment -2- | 230 | 224 |
| Me -3- | Methylated pigment -3- | 249 | 242 |
| Me Lignin 6 (*) | Corn cobs | 2 | 1 |
| Me Lignin 25 (*) | Marshall clay loam | 9 | 8 |

(*) Data from reference (61) (√) Data from reference (33)

From Table 3 we note that acetylation has reduced the base exchange capacity of each pigment only very slightly. Pigment -2-, which was acetylated the most readily and to the greatest extent, had its base exchange capacity affected the least by the increase in acetyl content. The decrease in exchange capacity of all three pigments is many times less than equivalent to the increase in acetyl content.

If the base exchange reaction of lignin and closely related substances is due primarily to phenolic hydroxyl groups, as suggested by various workers, some of the hydroxyls must not be esterified by acetic anhydride under the conditions used in this experiment. But since the pigments were acetylated readily there must be hydroxyl or other groups that may readily be esterified. These may be enolic, alcoholic, or very weakly acidic phenolic hydroxyl groups which play no part in the base exchange reaction.

Similarly, methylation decreased the base exchange capacity of all three pigments, but to a somewhat greater degree than did acetylation. Again, the loss in base exchange capacity of the pigments followed in the same order as it did for acetylation. That is, the loss was greatest for P1, next came P3, and the smallest loss of exchange capacity occurred in P2.

## TITRATIONS

It has been pointed out that this black pigment fraction of the soil organic matter has variously been referred to as humic acid, ulmic acid,

mull acid, et cetera. The question then naturally arises, does this black pigment behave as an acid or are the acidic properties ascribed to it by early workers merely due to absorption, or the presence of impurities? The presence of a carboxyl group and possibly phenolic groups has been suggested. Therefore conductometric and potentiometric titrations of the pigments might shed some light upon these questions.

The presence of hydrogen ion in humic acid was illustrated by Oden.(40) The concentration of hydrogen ion in his humic acid prepared from peat was equivalent to pH 3.87. The conductance of ammonium hydroxide was increased when he added humic acid. Therefore he concluded it was a true acid. He followed the neutralization of humic acid by means of conductivity measurements and found an approximate equivalent weight of 339. From his experiments he concluded it was a tetrabasic acid of medium strength.

The equivalent weight has been reported by various investigators, no two of which agree. They range from 147, 153 to 218 and 308. According to Oden the equivalent weight of humic acid, base exchange capacity at point of neutrality, is 330.

In this investigation the conductometric and potentiometric titrations were carried out in the following manner. One gram of the pigment, on an oven-dry ash-free basis, was placed in each of four 250 cc erlenmeyer flasks. Seventy-five cubic centimeters of carbon dioxide-free water was added to each. Flask number one, containing the pigment and distilled water, was set aside. Four, eight, and twelve cubic centimeters of 0.10N sodium hydroxide were added to flasks number two, three, and four, respectively. In addition, each flask contained five drops of toluene. These were then placed in the mechanical shaker for 24 hours. At the end

of this period the reaction, (pH), and the resistance, were determined by a pH electrometer, and a salt bridge, respectively. It was found that equilibrium was not attained until the solutions were shaken for a period of 20 hours or more. One cubic centimeter of 0.10N sodium hydroxide was added to each flask from a burette, shaken for 24 hours and the reaction and resistance again determined. This procedure was repeated daily until 4 cubic centimeters or more of sodium hydroxide had been added to each suspension. This same procedure was used on all three pigments, methylated pigments, and acetylated pigments. Sodium hydroxide was used in every titration. The conductivity measurements did not give very good results, and various attempts were made to sharpen any indicated end points. For example, one sample of pigment was leached with sulfuric acid, washed with water and titrated with barium hydroxide. The curve obtained by plotting resistances against cubic centimeters of base was not improved, and the end points in the potentiometric curve were less distinct than those obtained by titration with sodium hydroxide, in fact, the curve was almost a straight line. See Fig. 4. The barium ion evidently flocculated the pigment to such an extent that the reaction could not take place.

In an attempt to check on certain breaks in the potentiometric curves definite volumes (25 to 35 cc) of .10N sodium hydroxide were placed in small erlenmeyer flasks and minute amounts of the respective solid pigments were added to each flask. The suspensions were mechanically shaken for 24 hours after each addition of pigment. The results are shown in Fig. 6.

All titration curves are given in the following graphs.

CONDUCTOMETRIC TITRATION
of
Pigment -1- with 0.10N NaOH

| cc .10N NaOH | R | cc .10N NaOH | R | Dilution Curve cc .10N NaOH | R |
|---|---|---|---|---|---|
| 0.0 | 403 | 19 | 132 | 0.0 | > 10000 |
| 1 | --- | 20 | 134 | 1 | 650 |
| 2 | 388 | 21 | 131 | 2 | 330 |
| 3 | --- | 22 | 130 | 3 | 223 |
| 4 | 356 | 23 | 126 | 4 | 171 |
| 5 | 335 | 24 | 123 | 5 | 145 |
| 6 | 270 | 25 | 120 | 6 | 125 |
| 7 | 242 | 26 | 110 | 7 | 106 |
| 8 | 223 | 27 | 109 | 8 | 98 |
| 9 | 228 | 28 | 102 | 9 | 90 |
| 10 | 208 | 29 | 109 | 10 | 80 |
| 11 | 193 | 30 | 107 | 20 | 45 |
| 12 | 188 | 31 | --- | | |
| 13 | 186 | 32 | 93 | | |
| 14 | 176 | 33 | -- | | |
| 15 | 160 | 34 | -- | | |
| 16 | 154 | 35 | 85 | | |
| 17 | 151 | 36 | 80 | | |
| 18 | 134 | | | | |

CONDUCTOMETRIC TITRATION
of
Pigment -2- with 0.10N NaOH

| cc .10N NaOH | R | cc .10N NaOH | R |
|---|---|---|---|
| 0 | 400 | 19 | 136 |
| 1 | —— | 20 | --- |
| 2 | 388 | 21 | 131 |
| 3 | 373 | 22 | --- |
| 4 | 369 | 23 | 126 |
| 5 | 350 | 24 | 122 |
| 6 | 334 | 25 | 116 |
| 7 | 300 | 26 | --- |
| 8 | 285 | 27 | 111 |
| 9 | 248 | 28 | 98 |
| 10 | 221 | 29 | 103 |
| 11 | 208 | 30 | 103 |
| 12 | 200 | 31 | --- |
| 13 | 191 | 32 | 96 |
| 14 | 182 | 33 | 90 |
| 15 | 168 | 34 | 88 |
| 16 | 153 | 35 | 88 |
| 17 | 147 | 36 | 85 |
| 18 | 139 | | |

CONDUCTOMETRIC TITRATION
of
Pigment -3- with 0.10N NaOH

| cc .10N NaOH | R | cc .10N NaOH | R |
|---|---|---|---|
| 0.0 | 1160 | 14 | 160 |
| 1 | 1134 | 15 | — |
| 2 | 848 | 16 | 140 |
| 3 | 644 | 17 | 131 |
| 4 | 570 | 18 | 130 |
| 5 | 440 | 19 | 125 |
| 6 | 350 | 20 | 118 |
| 7 | 344 | 21 | 113 |
| 8 | 290 | 22 | 109 |
| 9 | 280 | 23 | 102 |
| 10 | 252 | 24 | — |
| 11 | — | 25 | 100 |
| 12 | 205 | 26 | — |
| 13 | 178 | 27 | 93 |

POTENTIOMETRIC TITRATION
of
Pigment -1- with 0.10N NaOH

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 4.05 | 23 | 6.52 |
| 1 | 4.20 | 24 | 6.79 |
| 2 | 4.30 | 25 | 6.91 |
| 3 | 4.48 | 26 | 7.12 |
| 4 | 4.59 | 27 | 7.30 |
| 5 | 4.65 | 28 | 7.55 |
| 6 | 4.65 | 29 | 7.81 |
| 7 | 5.00 | 30 | 8.00 |
| 8 | 5.08 | 31 | 8.10 |
| 9 | 5.05 | 32 | 8.18 |
| 10 | 5.11 | 33 | 8.20 |
| 11 | 5.12 | 34 | 8.24 |
| 12 | 5.21 | 35 | 8.29 |
| 13 | 5.31 | 36 | 8.45 |
| 14 | 5.30 | 37 | 8.71 |
| 15 | 5.41 | 38 | 8.90 |
| 16 | 5.58 | 39 | 9.02 |
| 17 | 5.61 | 40 | 9.05 |
| 18 | 5.85 | 41 | 9.10 |
| 19 | 5.92 | 42 | 9.30 |
| 20 | 6.08 | 43 | 9.31 |
| 21 | 6.29 | 44 | 9.39 |
| 22 | 6.40 | | |

POTENTIOMETRIC TITRATION
of
Methylated -l- with 0.10N NaOH

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.5 | 4.00 | 10.5 | 5.70 |
| 1.5 | 4.49 | 11.5 | 6.10 |
| 2.5 | 4.39 | 12.5 | 6.50 |
| 3.5 | 4.40 | 13.5 | 6.85 |
| 4.5 | 4.59 | 14.5 | 7.50 |
| 5.5 | 4.70 | 15.5 | 8.18 |
| 6.5 | 4.61 | 16.5 | 9.10 |
| 7.5 | 5.10 | 17.5 | 9.70 |
| 8.5 | 5.18 | 18.5 | 10.20 |
| 9.5 | 5.41 | 19.5 | 10.40 |

POTENTIOMETRIC TITRATION
of
Acetylated -l- with 0.10N NaOH

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.89 | 23 | |
| 1 | 4.00 | 24 | 6.38 |
| 2 | 4.08 | 25 | 6.50 |
| 3 | 4.10 | 26 | 6.60 |
| 4 | 4.13 | 27 | 6.70 |
| 5 | 4.30 | 28 | 6.80 |
| 6 | 4.90 | 29 | 6.90 |
| 7 | 5.06 | 30 | 6.85 |
| 8 | 5.16 | 31 | 7.05 |
| 9 | 5.25 | 32 | 7.15 |
| 10 | 5.39 | 33 | 7.18 |
| 11 | 5.49 | 34 | 7.35 |
| 12 | 5.50 | 35 | 7.44 |
| 13 | 5.59 | 36 | 7.40 |
| 14 | 5.69 | 37 | 7.55 |
| 15 | 5.75 | 38 | 7.85 |
| 16 | 5.74 | 39 | 7.90 |
| 17 | 5.89 | 40 | 7.88 |
| 18 | 5.91 | 41 | 8.10 |
| 19 | 5.99 | 42 | 8.35 |
| 20 | 6.00 | 43 | |
| 21 | 6.06 | 44 | |
| 22 | 6.26 | | |

POTENTIOMETRIC TITRATION
of
Pigment -2- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|---|---|
| 0.0 | 3.65 | 18 | 6.19 | 35 | 8.75 |
| 1 | 3.70 | 19 | --- | 36 | 8.85 |
| 2 | 3.70 | 20 | 6.39 | 37 | 8.95 |
| 3 | 3.90 | 21 | 6.60 | 38 | 9.16 |
| 4 | 4.15 | 22 | 6.73 | 39 | 9.49 |
| 5 | 4.40 | 23 | 6.89 | 40 | 9.20 |
| 6 | 4.62 | 24 | 7.12 | 41 | 9.30 |
| 7 | 5.15 | 25 | 7.25 | 42 | 9.30 |
| 8 | 5.10 | 26 | 7.35 | 43 | 9.39 |
| 9 | 5.22 | 27 | 7.49 | 44 | 9.50 |
| 10 | 5.39 | 28 | 7.71 | 45 | 9.56 |
| 11 | 5.42 | 29 | 8.04 | 46 | 9.62 |
| 12 | 5.47 | 30 | 8.11 | 47 | 9.70 |
| 13 | 5.50 | 31 | 8.19 | 48 | 9.76 |
| 14 | 5.50 | 32 | 8.39 | 49 | 9.93 |
| 15 | 5.60 | 33 | 8.50 | 50 | 10.00 |
| 16 | 5.81 | 34 | 8.66 | | |
| 17 | 6.00 | | | | |

POTENTIOMETRIC TITRATION
of
Pigment -2- with barium hydroxide

| cc .23N $Ba(OH)_2$ | pH | cc .23N $Ba(OH)_2$ | pH |
|---|---|---|---|
| 0.0 | 3.30 | 15 | 7.35 |
| 1 | 3.45 | 16 | 7.60 |
| 2 | 3.65 | 17 | 7.58 |
| 3 | 3.85 | 18 | 8.18 |
| 4 | 4.23 | 19 | 8.20 |
| 5 | 4.49 | 20 | 8.26 |
| 6 | 4.85 | 21 | 8.55 |
| 7 | 5.11 | 22 | 8.80 |
| 8 | 5.38 | 23 | 9.14 |
| 9 | 5.69 | 24 | 9.28 |
| 10 | 6.00 | 25 | 9.61 |
| 11 | 6.11 | 26 | 9.79 |
| 12 | 6.58 | 27 | 10.00 |
| 13 | 6.71 | 28 | 10.32 |
| 14 | 7.09 | | |

POTENTIOMETRIC TITRATION
of
Methylated -2- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.16 | 11 | 6.00 |
| 1 | 4.19 | 12 | 6.40 |
| 2 | 4.46 | 13 | 6.79 |
| 3 | 4.40 | 14 | 7.39 |
| 4 | 4.51 | 15 | 8.05 |
| 5 | 4.75 | 16 | 9.0 |
| 6 | 4.68 | 17 | 9.50 |
| 7 | 5.01 | 18 | 10.00 |
| 8 | 5.09 | 19 | 10.22 |
| 9 | 5.35 | 20 | 10.50 |
| 10 | 5.60 | | |

POTENTIOMETRIC TITRATION
of
Acetylated -2- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.69 | 17 | 6.06 |
| 1 | 3.89 | 18 | 6.21 |
| 2 | 4.00 | 19 | 6.31 |
| 3 | 4.10 | 20 | 6.38 |
| 4 | 4.10 | 21 | 6.50 |
| 5 | 4.25 | 22 | 6.60 |
| 6 | 4.31 | 23 | 6.66 |
| 7 | 4.71 | 24 | 6.90 |
| 8 | 5.36 | 25 | 7.00 |
| 9 | 5.39 | 26 | 7.03 |
| 10 | 5.50 | 27 | 7.14 |
| 11 | 5.55 | 28 | 7.24 |
| 12 | 5.65 | 29 | 7.36 |
| 13 | 5.71 | 30 | 7.37 |
| 14 | 5.90 | 31 | 7.45 |
| 15 | 5.95 | 32 | 7.50 |
| 16 | 5.99 | | |

POTENTIOMETRIC TITRATION
of
Pigment -3- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.70 | 24 | 6.60 |
| 1 | 3.89 | 25 | 6.80 |
| 2 | 4.05 | 26 | 6.98 |
| 3 | 4.30 | 27 | 7.29 |
| 4 | 4.60 | 28 | 7.30 |
| 5 | 4.80 | 29 | 7.60 |
| 6 | 4.89 | 30 | 7.98 |
| 7 | 5.09 | 31 | 8.00 |
| 8 | 5.21 | 32 | 8.16 |
| 9 | 5.20 | 33 | 8.30 |
| 10 | 5.20 | 34 | 8.50 |
| 11 | 5.21 | 35 | 8.62 |
| 12 | 5.25 | 36 | 8.65 |
| 13 | 5.30 | 37 | 8.85 |
| 14 | 5.30 | 38 | 8.90 |
| 15 | 5.40 | 39 | 9.10 |
| 16 | 5.42 | 40 | 9.13 |
| 17 | 5.51 | 41 | 9.38 |
| 18 | 5.70 | 42 | 9.30 |
| 19 | 6.00 | 43 | 9.40 |
| 20 | 6.35 | 44 | 9.36 |
| 21 | ---- | 45 | 9.50 |
| 22 | 6.35 | | |
| 23 | 6.41 | | |

POTENTIOMETRIC TITRATION
of
Methylated -3- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.56 | 14 | 6.70 |
| 1 | 4.45 | 15 | 7.20 |
| 2 | 4.60 | 16 | 7.61 |
| 3 | 4.58 | 17 | 8.22 |
| 4 | 4.59 | 18 | 8.90 |
| 5 | 4.74 | 19 | 9.40 |
| 6 | 4.71 | 20 | 9.70 |
| 7 | 4.89 | 21 | 10.00 |
| 8 | 5.25 | 22 | 10.11 |
| 9 | 5.37 | 23 | 10.45 |
| 10 | 5.60 | 24 | 10.65 |
| 11 | 5.71 | 25 | 11.00 |
| 12 | 6.00 | | |
| 13 | 6.39 | | |

POTENTIOMETRIC TITRATION
of
Acetylated -3- with sodium hydroxide

| cc .10N NaOH | pH | cc .10N NaOH | pH |
|---|---|---|---|
| 0.0 | 3.69 | 21 | 6.64 |
| 1 | 4.09 | 22 | 6.71 |
| 2 | 4.19 | 23 | 6.80 |
| 3 | 4.50 | 24 | 6.88 |
| 4 | 5.19 | 25 | 6.90 |
| 5 | 5.40 | 26 | 7.00 |
| 6 | 5.51 | 27 | 7.10 |
| 7 | 5.60 | 28 | 7.06 |
| 8 | 5.76 | 29 | 7.06 |
| 9 | 5.80 | 30 | 7.10 |
| 10 | 6.00 | 31 | 7.10 |
| 11 | 5.95 | 32 | 7.20 |
| 12 | 6.06 | 33 | 7.21 |
| 13 | 6.07 | 34 | 7.28 |
| 14 | 6.15 | 35 | 7.50 |
| 15 | 6.15 | 36 | 7.50 |
| 16 | 6.20 | 37 | 7.60 |
| 17 | 6.25 | 38 | 7.62 |
| 18 | 6.30 | 39 | ---- |
| 19 | 6.50 | 40 | 7.90 |
| 20 | 6.50 | | |

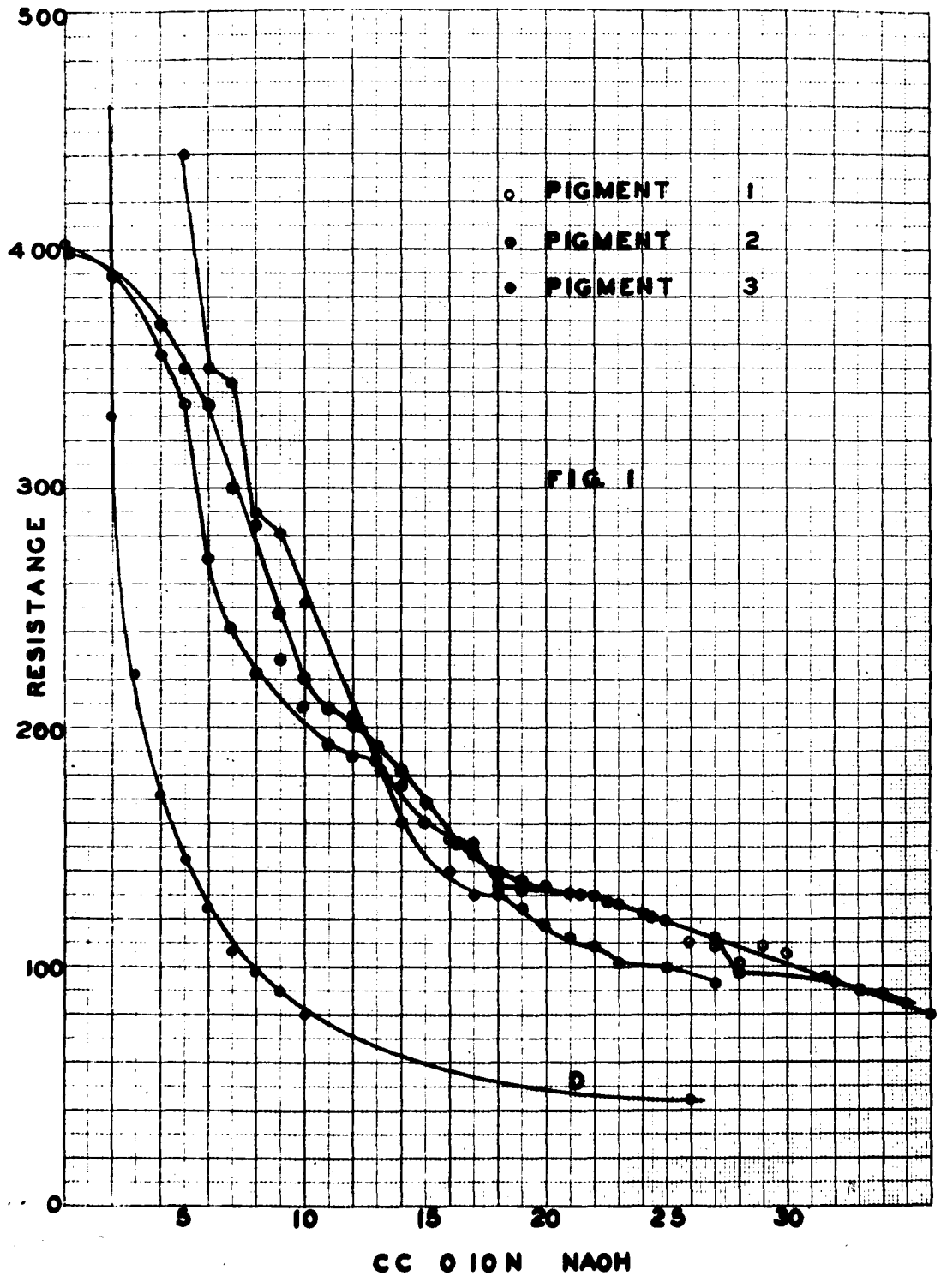

500
400
300
200
100
0
RESISTANCE
5
10
15
20
25
30
CC 0 10 N NAOH
o PIGMENT 1
• PIGMENT 2
• PIGMENT 3
D

FIG. 1

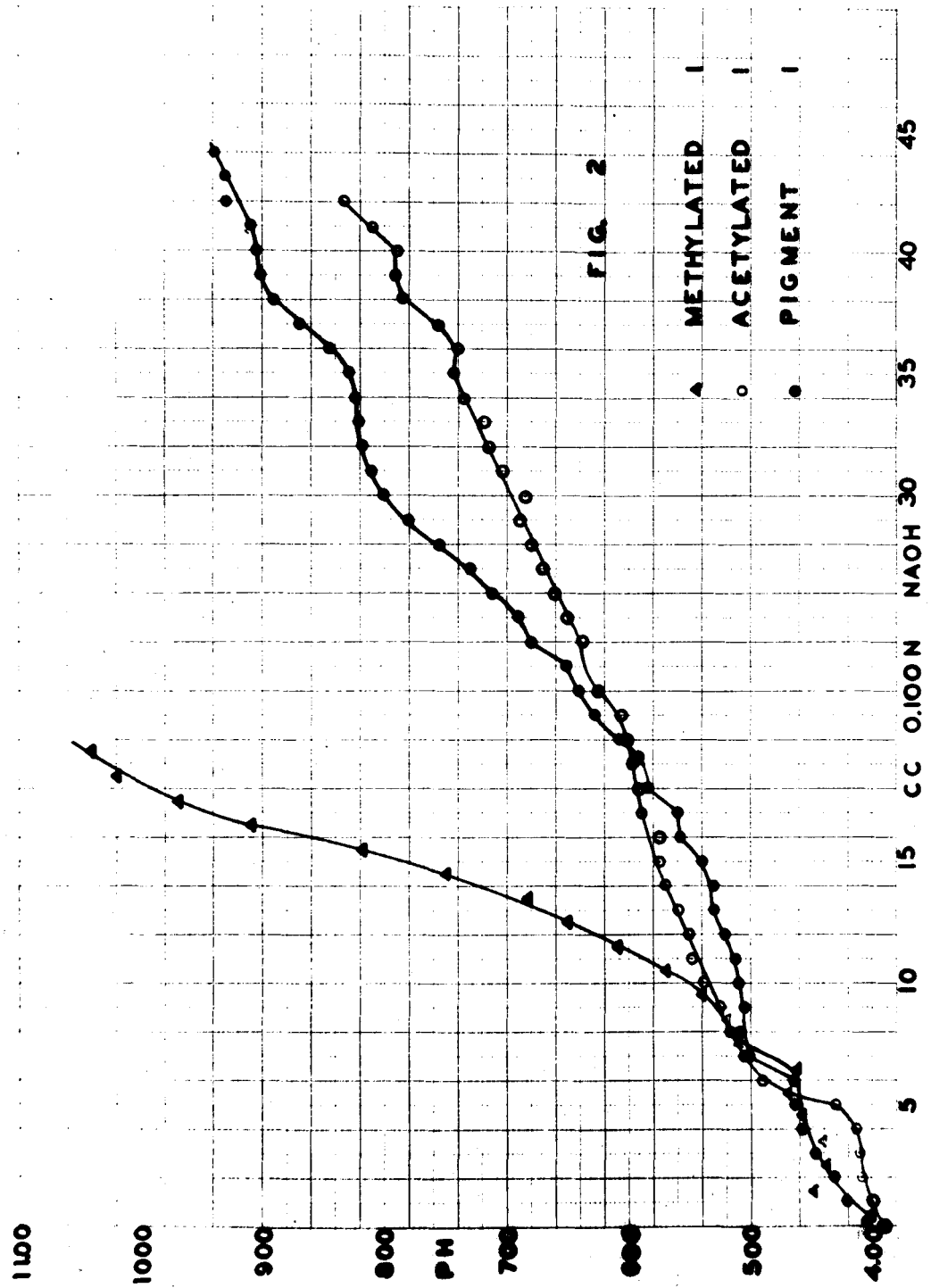


FIG. 2
METHYLATED
ACETYLATED
PIGMENT
1100
1000
900
800
700
600
500
400
MM
0
5
10
15
CC 0.100 N NAOH
30
35
40
45

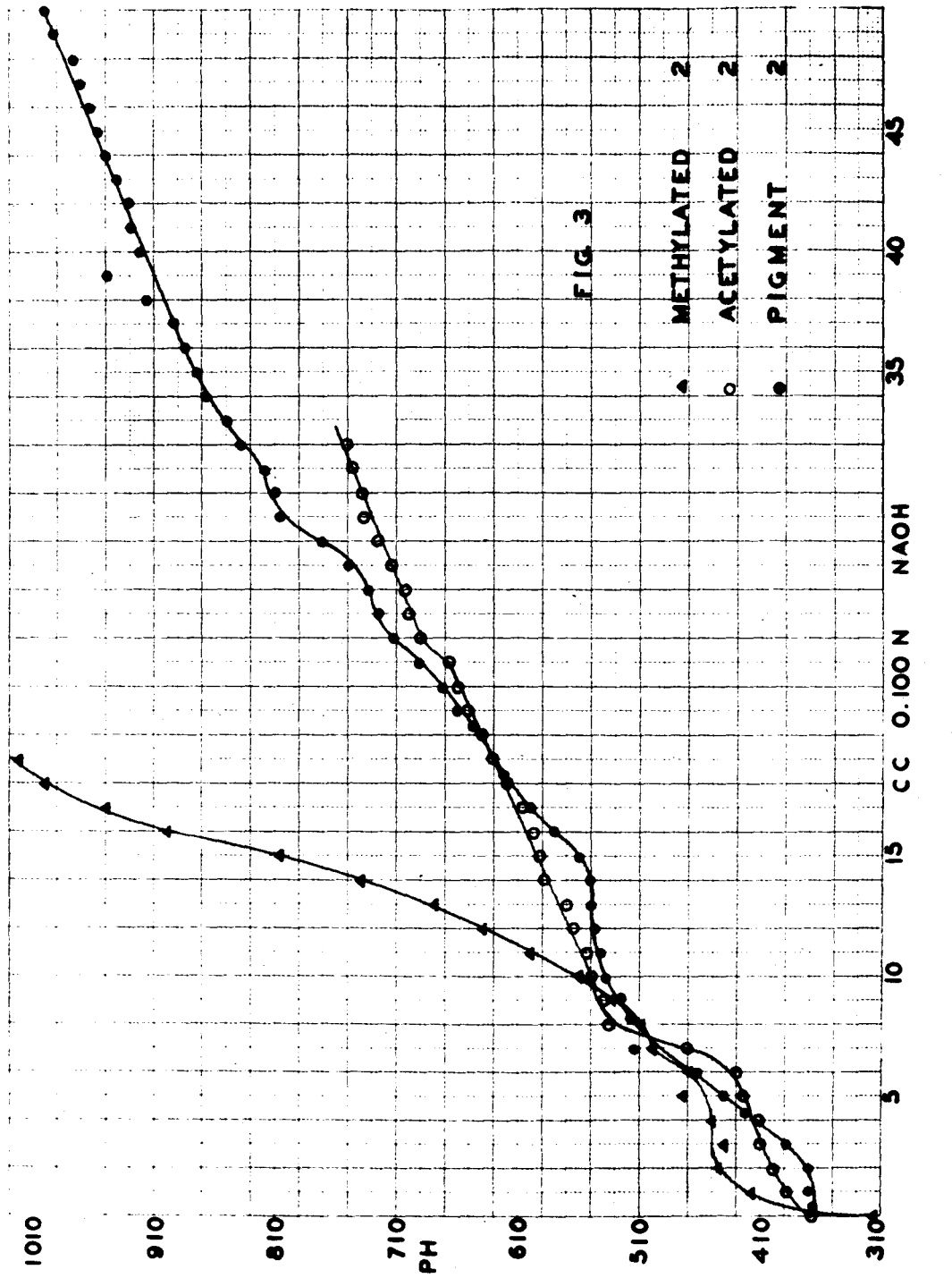


FIG 3
METHYLATED 2
ACETYLATED 2
PIGMENT 2
PH
1010
910
810
710
610
510
410
310
5
10
15
CC 0.100 N NAOH
33
40
43

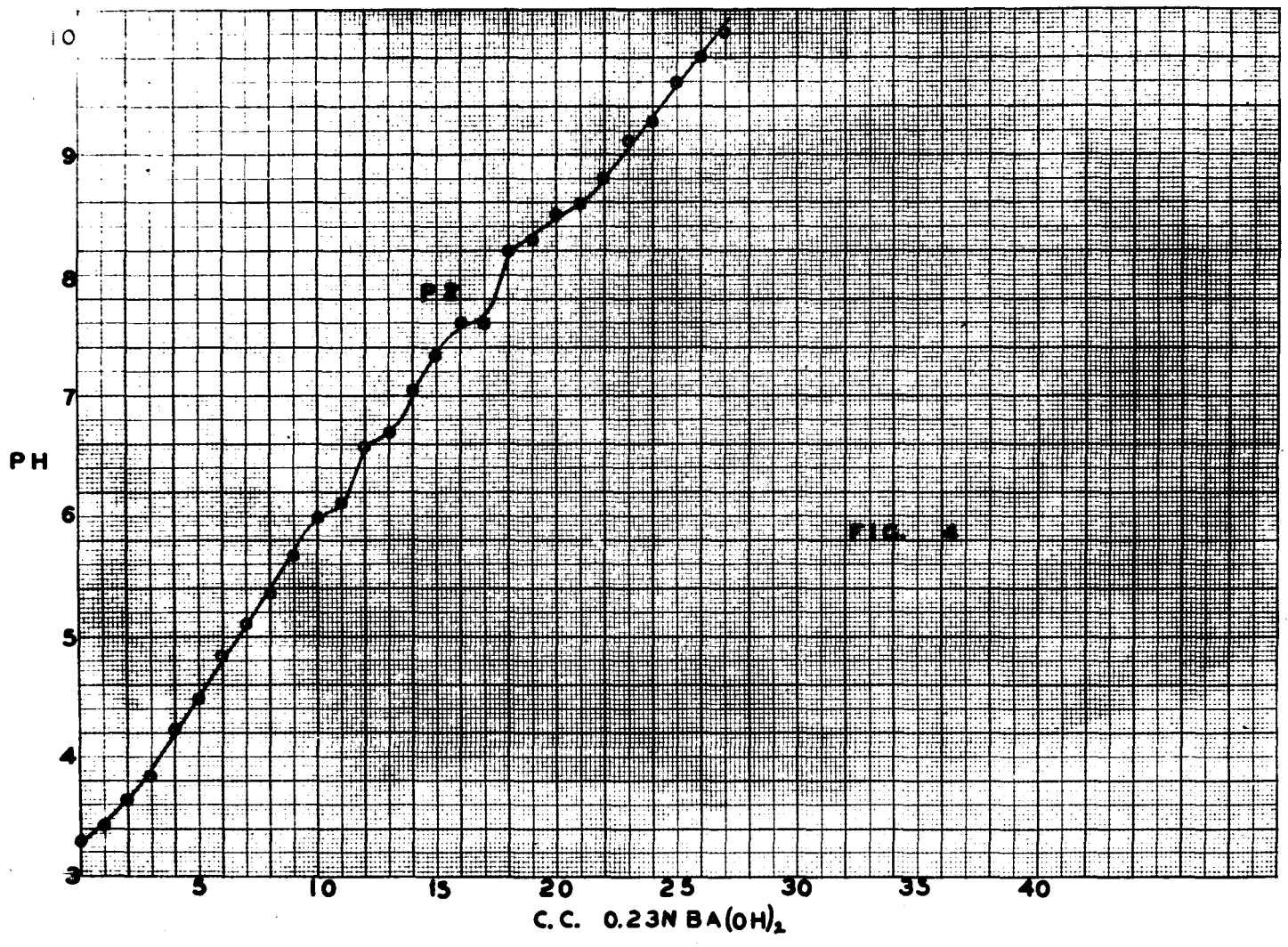



Fig. 8

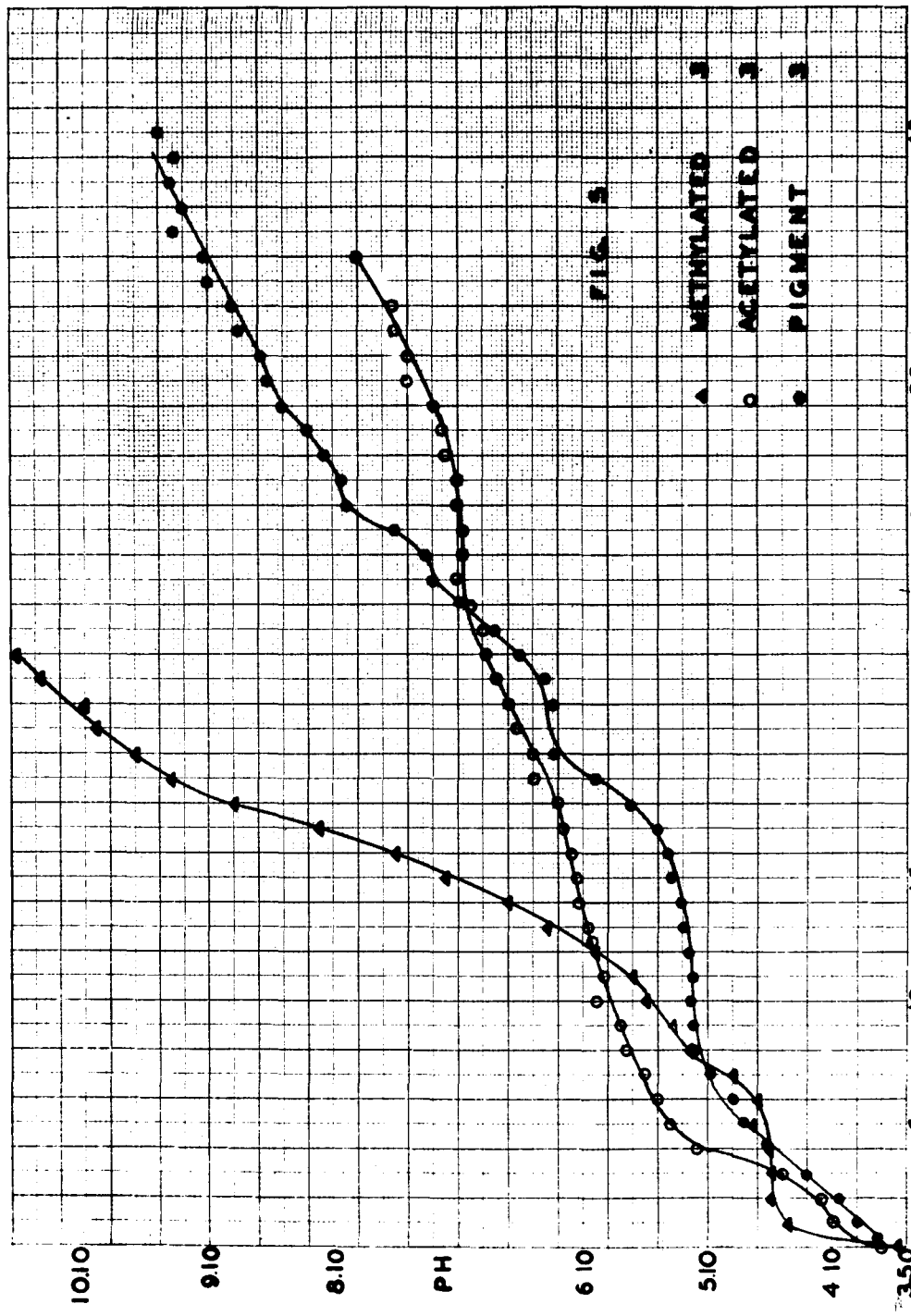
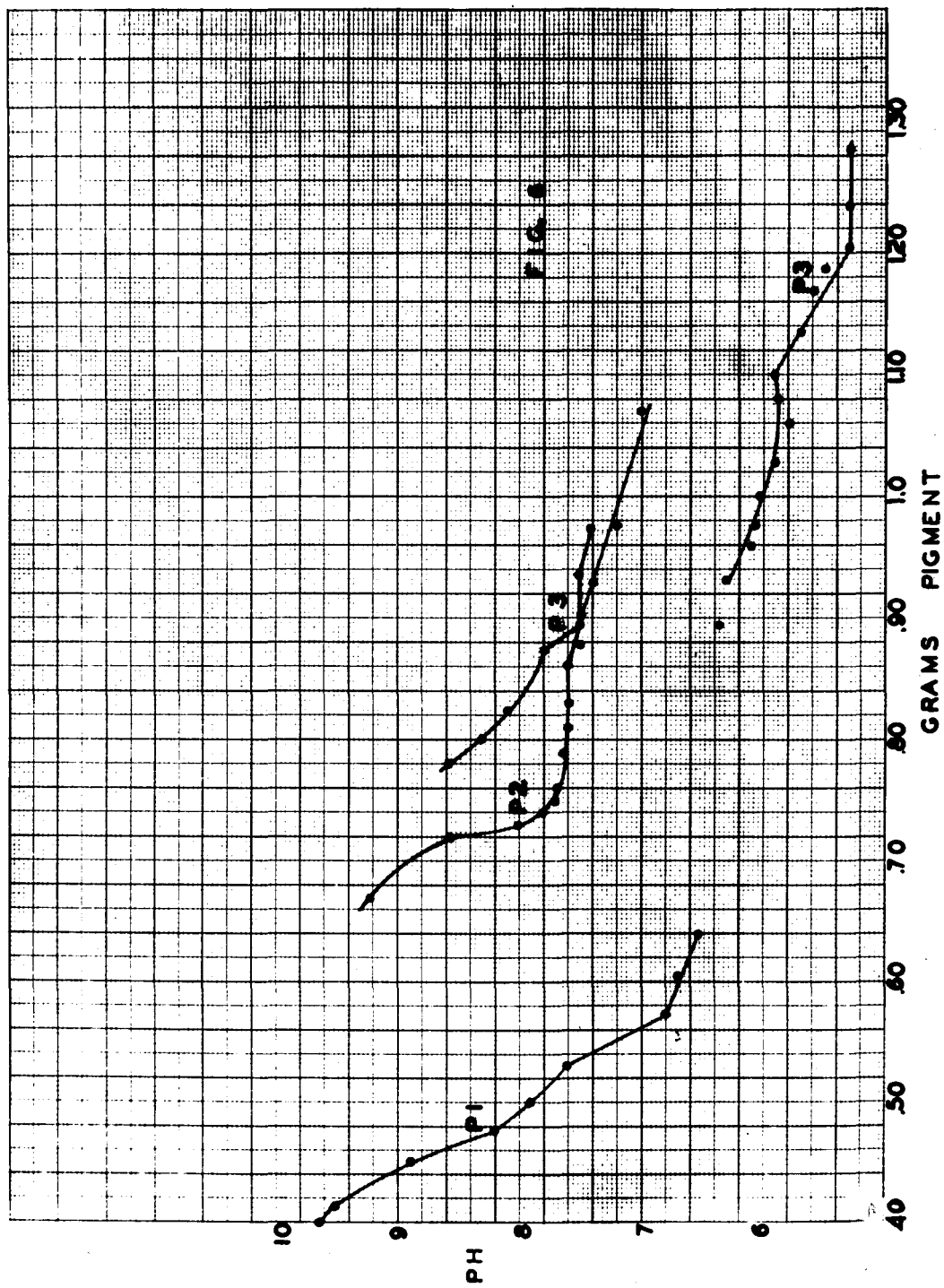
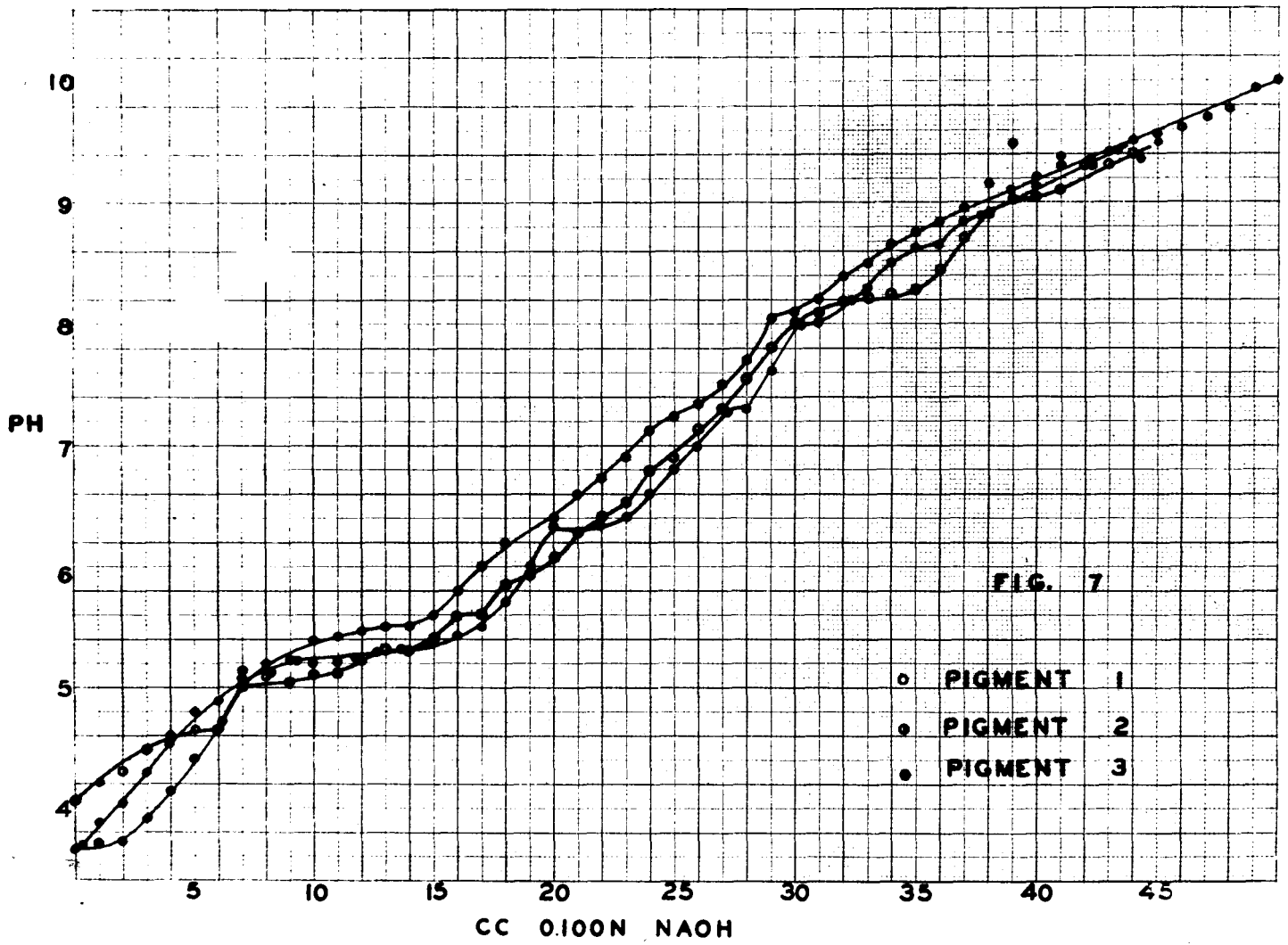
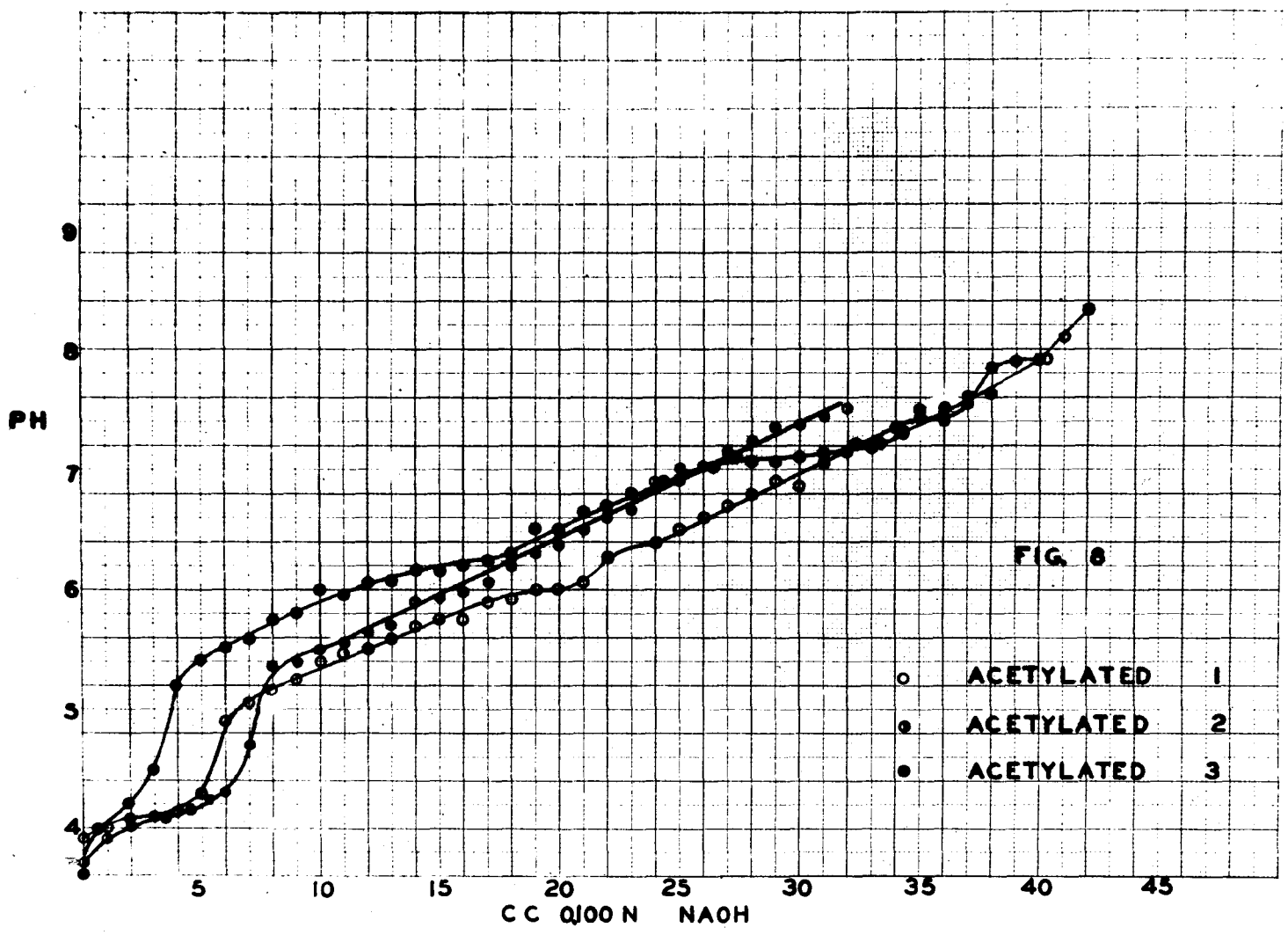


FIG. 5
METHYLATED
ACETYLATED
PIGMENT
PH
10.10
9.10
8.10
6.10
5.10
4.10
3.50
5
10
15
40
45
C C 0.100 N NAOH 30
35


PH
10
9
8
7
6
40
50
60
70
80
90
1.0
GRAMS PIGMENT
P1
P2
P3
P4


10
9
8
PH
7
6
5
4
5
10
15
20
25
30
35
40
45
CC 0.100N NAOH
FIG. 7
PIGMENT 1
PIGMENT 2
PIGMENT 3


PH
9
8
7
6
5
4
5
10
15
20
25
30
35
40
45
CC 0100 N NAOH
FIG. 8
ACETYLATED 1
ACETYLATED 2
ACETYLATED 3

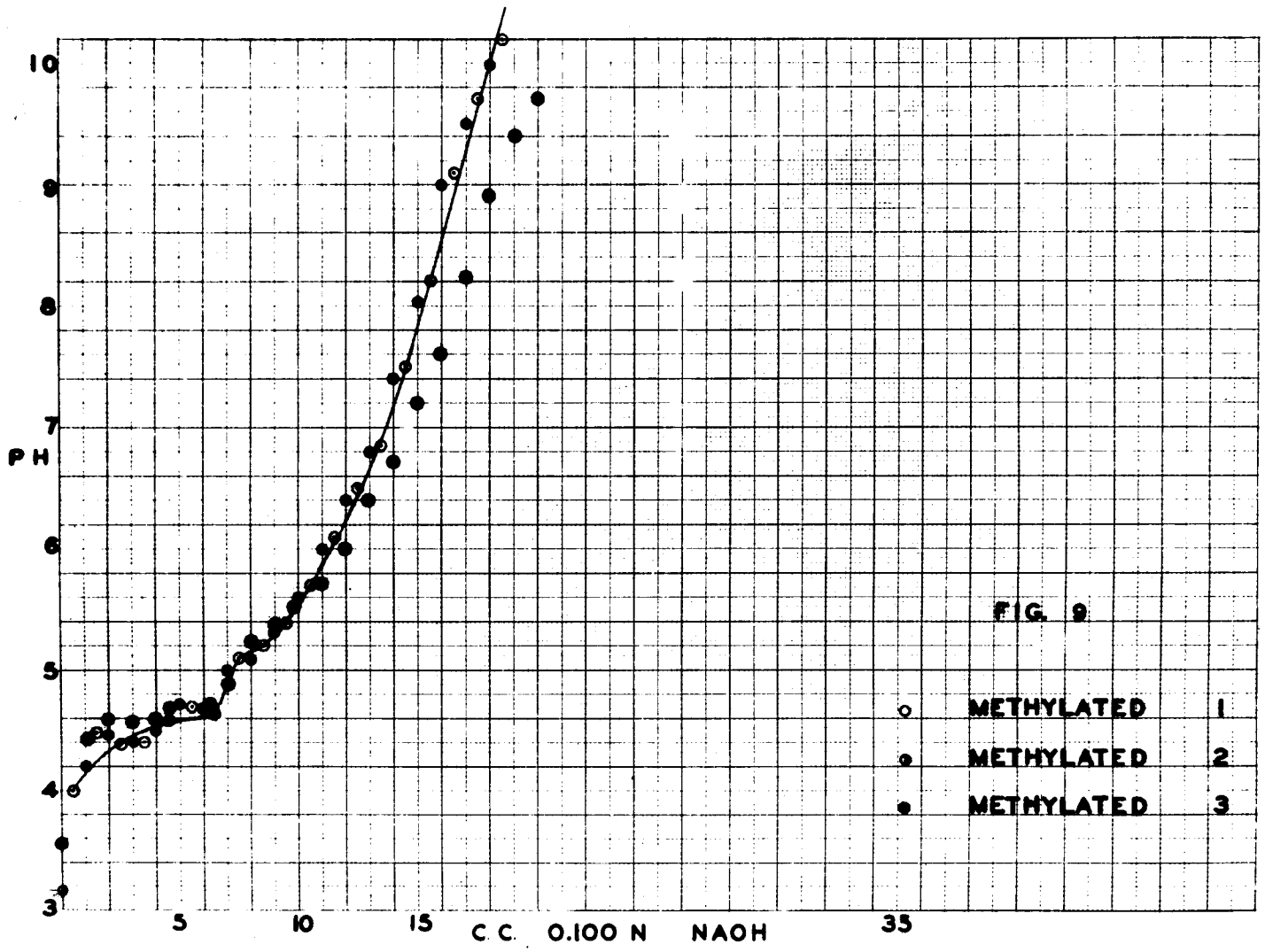

10
9
8
7
PH
6
5
4
3
5
10
15
C.C. 0.100 N NAOH
35
METHYLATED 1
METHYLATED 2
METHYLATED 3

FIG. 9

Figure 1 shows the type of curve obtained when the black pigment is titrated with .10N sodium hydroxide and resistance is plotted against cc base. In general all three curves indicate that the base is reacting with something, undoubtedly hydrogen ion. (D) represents the dilution curve, obtained by adding base in 1 cc portions to 75 cc of distilled carbon dioxide-free water.

The curve for pigment -1- apparently has two distinct breaks, which therefore indicates an end point at approximately 6 or 6.5 cc and one at 17.5 cc. Curve P2 has an approximate end point at 7 cc, and another more definite break at 27.5 cc. Finally, the titration curve of pigment -3- shows an end point at about 7.5 cc and an indication of one around 19 cc.

The potentiometric curves for pigment -1-, acetylated -1-, and methylated -1-, are plotted in Fig. 2. All three curves give very definite breaks between a pH of 4.2 and 5.00. The end point lies somewhere between 5.5 cc and 7 cc. The titration curve for pigment -1- has the following end points:

(1) 6.5 cc .10N NaOH; pH, 4.8

(2) (17.5 cc " " ; pH, 5.7)

(3) (23.5 cc " " ; pH, 6.7)

(4) 36.5 cc " " ; pH, 8.5

Breaks (2) and (3) are questionable but seem to indicate end points. These same points are reflected in the curve of acetylated -1-. Although these two breaks are not very obvious, it should be pointed out that they are six to eight times as great as the experimental error involved in determining the pH. Apparently then, the breaks are significant. End points (1) and (2) were likewise illustrated by the curve for P1, Fig. 1.

Methylation has blocked off all the acidic groups except one. The methylated compound therefore above a pH of 5.1, has no buffering capacity and the curve ascends sharply.

Acetylation apparently was not nearly as effective in blocking off the acidic groups as was methylation. In the titration curve for acetylated -1- the same two end points, (1) and (4) are present, evidently due to the presence of some groups which could not be acetylated under the conditions of the experiment. The end points agree rather closely with those obtained in the titration of pigment -1-.

Curiously, acetylation increased the buffering capacity of the pigment. At pH 6.00 the Ac1 curve crosses the P1 curve and remains below the latter throughout the rest of the titration.

Fig. 3 shows the potentiometric curves for pigment -2-, methylated -2-, and acetylated -2-. As in Fig. 2 all three materials possess a definite end point at a relatively low pH, between 3.7 and 5.5. The end point occurs between 6 and 7 cc of NaOH. The titration curve for pigment -2- possesses the following end points:

(1) 6 cc 0.10N NaOH; pH 4.6

(2) 27.5 cc " " ; pH 7.6

It is barely possible an end point exists around 19 cc, pH 6.3 but this cannot be determined definitely from this one curve. It is quite probable that still a third end point occurs around 39 cc .10N NaOH, pH about 9.3.

The curve for pigment -2- is the only one which was not checked by a duplicate. However, out of the 25 or more titrations that were made whenever a curve similar to that of P2, around pH 9.1 was obtained, its duplicate practically always showed a definite end point. The curve P2, Fig. 4, however,

affords supporting evidence for this end point. In this curve we note a fairly definite end point at 17.5 cc 0.23N barium hydroxide, which is equivalent to 40 cc of .10N sodium hydroxide.

Curve P2,(Fig. 1) indicates end points at approximately 7 cc and 27.5 cc, 0.10N NaOH. This checks the end points (1) and (2), above, shown by the potentiometric curve, fairly closely.

The methylated -2- curve possesses a sharp end point at 6.5 cc, pH 4.8. The acetylated -2- curve shows a very pronounced end point at 7 cc pH 4.8, and one other break in the curve at 23.5 cc.

Again we see that acetylation has increased the buffer capacity of the pigment very slightly, above pH 6.4.

Fig. 4 shows the same pigment titrated with $Ba(OH)_2$.

The potentiometric curves for P3, Me3, and Ac3 are shown in Fig. 5. Curve P3, (Fig. 1) indicates definite end points at 7.5 cc and 19 cc. The end point for pigment -3- at a relatively low pH, is not nearly as definite as it is for the corresponding breaks in the titration curves of pigments -1- and -2-. However the methylated product shows a very definite end point at 7 cc, pH 5.0, and the curve for the acetylated pigment likewise indicates an end point at pH 4.8. The end points for pigment -3- indicated by the potentiometric curve are:

(1) 4 cc 0.10N NaOH, pH 4.6

(2) 18 cc " " , pH 5.7

(3) 26 cc " " , pH 7.1

Acetylation apparently has been fairly successful in blocking the groups responsible for the breaks in the curve at 18 and 26 cc, since the curve is relatively smooth.

The curves in Fig. 6, obtained by titrating .10N NaOH with solid pigment,

indicate definite end points within the range studied. For example, with pigment -1-, end points are indicated at pH 8.8 and 7.2, with pigment -2- a definite break occurs at pH 8.1, and for pigment -3- definite end points occur at pH 7.6 and 5.6.

The titration curves for the three pigments are plotted in Fig. 7. They show a marked similarity. All possess a definite end point at a relatively low pH, approximately 4.7. For pigments -1- and -2- this occurs at 6.5 cc and 6 cc respectively. With pigment -3- the titration curve gives no sharp break and the end point therefore is more or less of an approximation at 4 cc. Pigments -3- and -1- have their second end points at 18 and 17.5 cc of .10N NaOH, and their third end points occur at 26 cc and 23.5 cc respectively. In the same portion of the diagram P2 shows its second end point at 27.5 cc of 0.10N NaOH. Finally, a fourth end point occurs with pigment -1- at 36.5 cc, and possibly the third break for pigment -2- in the neighborhood of 39 cc.

The curves in Fig. 8 illustrate very well the definite end points occuring in the acetylated product. Acetylation did not destroy the pigments buffering capacity, but it did tend to eliminate, with a few exceptions, the other breaks found in the pigment titration curves.

Probably the most striking curve of all is shown in Fig. 9. This shows graphically that the methylated products of all three pigments are practically identical in their behavior towards titration. Since the pigments are probably synthesized from the same central nucleus and methylation has completely blocked most of the acidic groups, which were formed by partial degradation of the central nucleus, it is reasonable to suspect that the titration curves of the methylated products would be identical.

## DISCUSSION AND CONCLUSIONS

The soil black pigment has been extracted and studied rather extensively from three soil groups, namely, gray-brown forest soils, muck soil, and grassland soils of the Chernozem, Prairie, and Chestnut regions. The three pigments were found to be very similar in all physical properties. They are all colloidal in nature, peptized by dilute alkalies, and precipitated by cations, the same as other negatively charged colloids. Acids flocculate the suspensions of these pigments according to their degree of dissociation. The highly dissociated acids flocculate them the most completely. It was impossible to coagulate the pigments by using acetic acid. When alkaline suspensions of the pigments were placed under the influence of an applied e. m. f. they migrated towards the anode, demonstrating that they are negatively charged. Suspensions of the pigments in water, pyridine, sodium hydroxide, ammonium hydroxide, alcohol, et cetera, gave distinct Tyndall cones. Furthermore, the light reflected from these cones was polarized, conclusive evidence that the pigments do not form true solutions in the above solvents.

The freshly prepared pigment was very voluminous, due to adsorbed water, and when separated from the dispersion medium it formed an elastic gel. This shrank enormously on drying and eventually became a brittle solid. The process resembles somewhat the drying of silicic acid gel. When dry the pigment will not again form a sol upon the addition of water. However, it is a reversible colloid if kept moist.

Electrical conductivity of the pigments suspended in distilled water is very low, ranging from 400 to 2000 reciprocal ohms.

Apparently the black pigment exists in the soil solution as a hydrophile colloid.

It has been demonstrated by various workers that nitrogen in the black pigment is present in a protein-like combination. Therefore, using the conventional protein factor 6.25, pigments -1-, -2-, and -3-, (Table 4), will respectively contain 37 per cent, 34 per cent, and 38 per cent of protein-like material and 63 per cent, 66 per cent, and 62 per cent of a different complex containing no nitrogen. By using an average figure for plant protein the composition of this remaining complex can be calculated, and is given in Table 4 directly under the three pigments.

Table 4

| | Per cent C | Per cent H | Per cent N | Per cent O |
|---|---|---|---|---|
| Pigment -1- | 58.35 | 4.54 | 5.88 | 31.2 |
| Pigment -2- | 58.81 | 5.54 | 5.49 | 30.2 |
| Pigment -3- | 55.58 | 5.17 | 6.03 | 33.2 |
| P -1- minus protein | 62.0 | 3.1 | 0.0 | 34.9 |
| P -2- " " | 62.3 | 4.8 | 0.0 | 32.9 |
| P -3- " " | 57.7 | 4.0 | 0.0 | 38.3 |
| Humic Acid (peat) (*) | 58.2 | 4.3 | 1.0 | 36.5 |
| " " (Soil)(*) | 56. | 5.1 | 5.4 | 33.5 |
| Plant protein(*) | 52. | 7.0 | 16. | 25.0 |
| Cellulose(*) | 44.4 | 6.2 | 0.0 | 49.4 |
| Lignin(*) | 69. | 6.0 | 0.0 | 25. |
| Lignin(x) | 61-64 | 5-6 | 0.0 | 30 (Approx.) |

(*) Data from reference (30) (x) Data from reference (39)

(The carbonyl content of humic acid reported in the literature varies from 0.4 per cent to 2.2 per cent.)

Comparing these fractions, containing no nitrogen, with cellulose and lignin we see that they are quite similar to lignin in so far as carbon and oxygen are concerned but are lower in content of hydrogen. Apparently there is a close relationship between the black pigments of the soil and lignin, a relationship which is also shown by certain chemical reactions.

Any differences between the three pigments are slight, and it was thought probable that these differences might be brought out by the chemical reactions used in this investigation. Acetylation established the fact that pigment -2- was most readily acetylated, followed by pigment -3- and pigment -1-. The relative amount of acetylation that took place followed the same order, Ac2 being the most completely acetylated product, followed by Ac3 and Ac1.

Compared to Ac1, both Ac2 and Ac3 contain a relatively much larger amount of acetic acid, (206 M. E. as compared to 313 and 308 M. E. respectively), (Table 1). This would indicate a difference in the number or nature of groups present in pigment -1- which were acetylated under the conditions employed. One might expect this difference to appear in the titration curves for the acetylated products. It is noted (Fig. 8) that Ac1 possesses a distinct break at a relatively high pH. Apparently P1 contains a group that was not acetylated by the procedure used and consequently the acetyl content of Ac1 might be expected to be lower than either Ac2 or Ac3.

Lignin isolated from a Marshall clay loam soil contained 1.5 per cent acetic acid, which is almost identical with the acetic acid content of the three pigments. However, it could be built up by acetylation to 22.9 per

cent, a higher content than was possible with any of the pigments. Although lignin isolated from corn cobs contained a very small percentage of acetic acid, 0.2 per cent, its content could be increased to 13.6 per cent. The pigments, on the other hand, were built up from around one per cent to 12 per cent and 18.8 per cent acetic acid.

The methoxyl contents of all three pigments are low, ranging from 1.03 per cent to 1.74 per cent. Similarly, the methoxyl content of lignin isolated from a marshall soil is low, being 1.6 per cent. Compare these values with the methoxyl contents of lignin derived ffom corn cobs and other plant materials, 12.9 per cent and 15 per cent respectively, and we note a decided difference. Apparently, during the formation of the black pigment from plant lignin loss of methoxyl groups took place to a considerable extent. The pigments extracted from forest and muck soils have a slightly higher methoxyl content than the pigment obtained from grassland soils. The total methoxyl content of plant lignins can be built up by methylation to a much greater degree than can soil lignin (Table 2). Likewise, the total methoxyl content of soil lignin can be increased to a greater extent than it can be in the soil black pigment. In the case of the former the methoxyl content can be increased from 1.6 per cent to 17.5 per cent but with the pigment it increases only from about 1.6 per cent to approximately 8.9 per cent.

It has been shown that methylation and acetylation decreased the base exchange capacity of all three pigments. The decrease however, is much less than equivalent to the increase in acetyl and methoxyl content. This clearly indicates the presence of hydroxyl or other groups, which readily undergo methylation and acetylation, but which are not involved in the base exchange reaction.

From Tables 1, 2, and 3 we note that corn cob lignin methylates and acetylates readily but its base exchange capacity is low. Apparently, corn cob lignin contains various groups which can be methylated and acetylated, but which do not undergo the base exchange reaction. The groups which are responsible for the base exchange properties, however, are also methylated and acetylated to a large degree, for the base exchange capacity was reduced to almost nil.

During the transformation of plant lignin into soil lignin, partial degradation probably took place. This consisted of demethylation, probably hydrolysis of certain groups, fission of some specific rings or linkages, and various other chemical reactions that would result in the formation of carboxyl or phenolic hydroxyl groups. These groups being active in the base exchange reaction would greatly increase the base exchange capacity of soil lignin. The complex, however, would still possess groups which could be methylated and acetylated but which would be inactive towards base exchange. Consequently, soil lignin should methylate and acetylate the most readily of all three complexes, (corn cob lignin, soil lignin and soil pigment). In other words, the <u>increase</u> in methoxyl and acetyl content should be greatest in the soil lignin. The data substantiates this assumption. For corn cob lignin, the increase in methoxyl and acetyl content was respectively, 491 and 223 M. E. per 100 grams of sample. Similarly, for soil lignin the increase of methoxyl and acetyl content was respectively, 547 and 356 M. E. per 100 grams and for soil pigment around 225 and 280 M. E. per 100 grams of sample. Likewise, the base exchange capacity of soil lignin was very high. Since the base exchange capacity was reduced tremendously by acetylation and methylation of soil

lignin, it is apparent that these reactions are centered, primarily, in the same groups that are responsible for the base exchange reaction.

During the final stage in the synthesis of the soil black pigment, the soil lignin has undergone still more modification and has combined in some way with a nitrogenous material. Since the decomposition of organic matter increases the base exchange capacity, one would expect soil lignin and soil pigment to possess a much higher exchange capacity than plant lignin. The base exchange capacities of the pigments are of the same order as that of the soil lignin, although in pigments -2- and -3- they are slightly lower than for the lignin.

Relative to soil lignin, the capacity for methylation and acetylation of the pigments has been decreased, but their capacity for acetylation has not been decreased to as low a value as that of the corn cob lignin. Therefore, during the formation of the pigment from soil lignin there has been a loss or rearrangement of certain groups, or possibly a linking of the non-nitrogenous portion to the protein-like complex through these groups, which therefore, has reduced the pigments' capacity to be methylated or acetylated, but has not greatly affected the groups responsible for base exchange.

With the soil pigment acetylation and methylation are not centered in groups active in the base exchange reaction as they are with soil lignin, but apparently react almost entirely with other groups. These may be alcoholic, enolic, or weakly acidic phenolic hydroxyl groups. Consequently, during this latter stage of pigment formation there probably has been only a very slight loss of carboxyl, hydroxyl, or other groups that are involved in base exchange, from the soil lignin molecule, but probably a greater loss of groups not concerned with the above reaction has occurred.

The decomposition of plant lignin when incorporated in the soil takes place very slowly. As it is modified it combines with a nitrogenous material, probably through the influence of microorganisms, to form the soil black pigment. Since the decomposition of plant lignin into soil lignin is slow, it allows sufficient time for practically all of the soil lignin to be used up in the synthesis of the soil pigment. Consequently, only a realtively small quantity of soil lignin is present in the soil, but a fairly large amount of soil pigment.

The non-nitrogenous portion of the soil black pigment probably consists of a modified soil lignin molecule.

The potentiometric curves bring out some striking similarities and a few differences between the three soil pigments. The titration curves for all three pigments possess a definite end point at a pH of approximately 4.6. Subsequent methylation and acetylation failed to eliminate the break in the curve. It seems fairly reasonable to assume that this end point is due to the presence of a carboxyl group.

Acetylation apparently has sharpened these end points around pH 4.6, since the breaks in the acetylated curves are much more pronounced than they are in the pigment curves. With the exception of Acl, acetylation tended to smooth the titration curves of all three pigments beyond pH 4.6. In other words, certain groups causing a break in the pigment curves have been blocked or partially blocked by esterification. The curve for Acl indicates that pigment -1- possesses acidic groups which are not readily acetylated by acetic anhydride under the conditions employed. In this respect it differs from P2 and P3. Furthermore, acetylation of the pigments had little effect on their buffering capacity. However, with

continued addition of sodium hydroxide the acetylated curves cross the pigment curves and the buffering capacities of the acetylated products become greater than those of the pigments.

If the base exchange reaction of organic materials is primarily due to specific groups, these groups might show up in the titration curve. For example, the curve for pigment -1- possesses four breaks, and it follows that if these are caused by carboxyl and hydroxyl groups then pigment -1- should possess the highest base exchange capacity, followed in order by pigment -2- and pigment -3-, each of which probably possesses three definite end points. Base exchange data gave the following values:

Pigment -1- = 396 M. E. Ba per 100 grams
" -2- = 292 " " " " "
" -3- = 239 " " " " "

showing a correlation between the presence of acidic groups and base exchange capacity.

Similarly, since the curve Ac1, Fig. 8, possesses two distinct breaks compared to one break, for Ac2 and Ac3 the base exchange capacity for acetylated -1- might be expected to be higher than for either of the other two. This is shown to be true by the data in Table 3.

A comparison of the potentiometric curves for all three pigments is given in Fig. 7. The end points for these curves are summarized below. The curves are strikingly similar, differing primarily in the number and location of their respective breaks.

| | Pigment | | | | | |
|---|---|---|---|---|---|---|
| | -1- | | -2- | | -3- | |
| End Point | cc 0.10N NaOH | pH | cc 0.10N NaOH | pH | cc 0.10N NaOH | pH |
| 1 | 6.5 * 6.5 | 4.8 | 7.0 * 6.0 | 4.6 | 7.5 * (4) ? | 4.6 |
| 2 | 17.5 * (17.5) | 5.7 | | | 19 * 18 | 5.7 |
| 3 | (23.5) | 6.6 | 27.5 * 27.5 | 7.6 | 26 | 7.1 |
| 4 | 36.5 | 8.5 | 39 | 9.3 (Approx.) | | |

(*) end point from conductometric measurements.

Methylation of the pigments succeeded in blocking all acidic groups except the carboxyl. The buffering capacity of the material has been reduced tremendously and the potentiometric curve rises sharply. If all three pigments were synthesized from the same central nucleus, e. g. lignin, potentiometric titrations of the methylated products should give practically identical curves, Fig. 9. Since the acidic group in pigment -1- at pH 8.6 was successfully blocked by methylation, but not by acetylation, it possibly indicates the presence of a phenolic hydroxyl group.

Titrations with $Ba(OH)_2$ were not successful since the barium ion flocculated the pigments to such an extent that the reaction could not proceed.

The absorption capacity of these pigments at neutrality can readily be calculated from the curves. The titration curves intersect a line drawn horizontally from pH 7.0 at a definite point, corresponding to a certain volume of .10N NaOH or to a given number of milliequivalents of NaOH per gram of pigment. The amount of absorbed base on a milliequivalent basis was found to agree fairly well with the exchange capacity for

the pigment and acetylated product.

| Sample | Volume 0.106N NaOH | Absorption Capacity M.E. Na per 100 g. | Base Exchange Capacity M. E. Ca per 100 g. |
|---|---|---|---|
| Pigment -1- | 25.5 cc | 270 | 392 |
| Pigment -2- | 23.5 cc | 249 | 256 |
| Pigment -3- | 25.6 cc | 271 | 267 |
| Acetylated -1- | 30.5 cc | 305 | 345 |
| Acetylated -2- | 25.2 cc | 252 | 250 |
| Acetylated -3- | 25. cc | 250 | 225 |
| Methylated -1- | 13.5 cc | 143 | 271 |
| Methylated -2- | 13.4 cc | 142 | 230 |
| Methylated -3- | 14.8 | 157 | 249 |

The variation between the absorption capacity and base exchange capacity for pigment -1- is possibly due to the fact that the pigment had not been leached sufficiently by dilute acid in order to replace all cations on the complex by hydrogen ions. There is, however, a wide discrepancy between these two values for the methylated products. The absorption capacity has been decreased over 100 M. E. per 100 grams by methylation. The decrease in absorption capacity for Me -1- is practically identical with the decrease in base exchange capacity. For Me -2- and -3-, however, the decrease in absorption capacity is very much greater than the decrease in base exchange capacity. It is not known whether this difference is due to adsorption of anions (OH or $C_2H_3O_2^-$), the formation of a highly ionized sodium micelle, in contrast to the slightly ionized calcium micelle, or to some other physical or chemical phenomenon.

## CONCLUSIONS

The black pigments isolated, are negatively charged hydrophilic colloids.

Combustion analysis shows their elementary compositions to be somewhat analogous to that of lignin.

The acetyl content of soil lignin is practically identical with that of the black pigment. Acetylation of both readily takes place.

Acetylation increased the buffering capacity of all three pigments.

The methoxyl content of all three pigments is low and very similar to that of soil lignin.

The pigments apparently possess a carboxyl group, as shown by potentiometric titrations.

The increase in acetyl content, due to acetylation of the pigments, was practically the same as the increase of methoxyl content by methylation. With plant and soil lignin the increase in methoxyl content is respectively 2 and 1½ times as great as the increase in acetyl content.

Soil lignin can be methylated and acetylated more readily and more completely than either corn cob lignin, or the black pigments.

All three pigments possess hydroxyl groups which can be esterified or methylated, but which do not undergo the base exchange reaction. These may be alcoholic, enolic, or weak phenolic groups.

Differences between the three pigments are very slight, and consist of the following:

1. Pigments -2- and -3-, from muck and gray-brown forest soils respectively, are more easily and more completely acetylated than pigment -1-, from grassland soils.

2. The potentiometric titration curves of the acetylated pigments show that pigment -1- possesses an acidic group, not found in the other two, which cannot be esterified by acetic anhydride, or it possesses a group common to the other pigments but whose nature differs greatly.

3. Pigments -2- and -3- have a slightly higher methoxyl content than pigment -1-.

4. Base exchange capacity is greatest for pigment -1-.

The slight differences noted probably are due to the presence or absence of certain minor groups in the complex.

The methylated products of all three pigments are very similar, indicating that the same central nucleus is present in each pigment.

The non-nitrogenous fraction of the pigment apparently consists of a modified lignin complex .

Plant lignin, when incorporated in the soil, is transformed into soil lignin which in turn is synthesized into soil pigment.

The amount of lignin present in any grassland soil is small compared to the amount of pigment in the soil.

During the synthesis of the black pigment from the lignin complex there was not only a loss of methoxyl groups from the lignin molecule, but also a decrease in its capacity to be methylated.

The absorption capacities of the pigments and acetylated pigments at pH 7 agree fairly well with the corresponding base exchange capacities.

THEORIES OF CONSTITUTION

A great variety of empirical formulas for humic acid have been advanced, a few are given below:

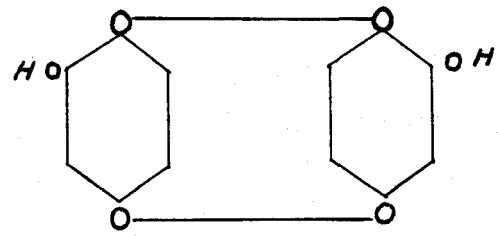
$C_{30}H_{30}O_{15}$ $C_{60}H_{54}O_{27}$

$C_{18}H_{14}O_6$ $C_{60}H_{52}O_{24}(COOH)_4$

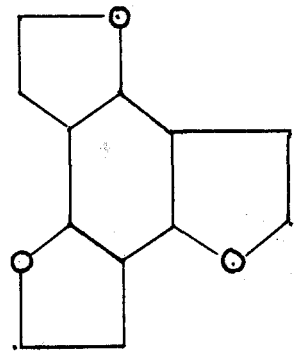
$C_{66}H_{52}O_{30}$ $C_{64}H_{52}O_{32}$

It appears that every investigator used a different name and a different empirical formula. However, it is not surprising that such a lack of uniformity existed when one realizes that each investigator was probably working with different fractions, that probably no pure chemical compound was isolated, and that the nature of the pigment or humic acid isolated may even depend upon the method used in its extraction.

Oden[41] prepared a synthetic humic acid having an elementary composition of C = 58.05 per cent, H = 3.25 per cent, by oxidation of phenol derivatives. It appeared to be identical with natural humic acid. He therefore proposed the following structural formula:

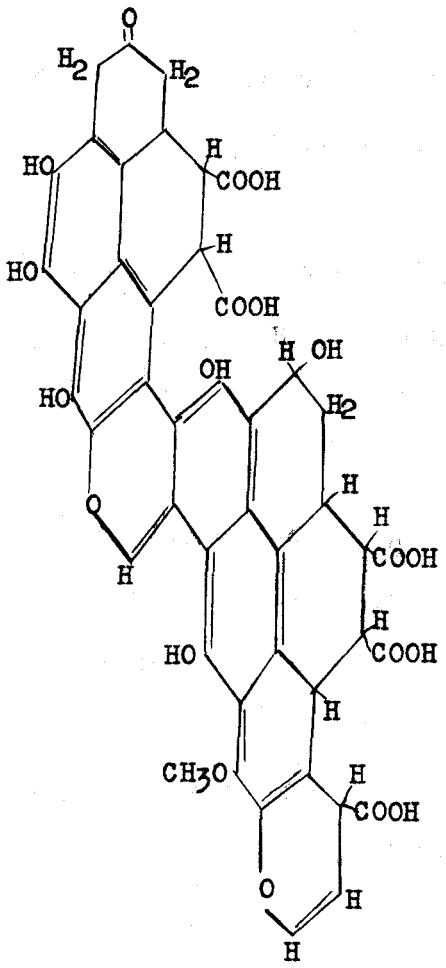



Another structural formula was proposed by Marcusson[28] as a result of his synthesizing a humus-like material from fural.



However, it is quite obvious that these two structural formulas will not account for the properties of the black pigment, nor for the presence of nitrogen.

Still another and more complex structural formula was advanced by Fuchs.[15]




This formula represents the pigment as possessing five carboxyl and several phenolic hydroxyl groups. Thus the pigment in certain respects should behave as a true acid. The hydroxyl and carboxyl groups present in such a unit as this would account for the high base exchange capacity of the pigment and for the loss of exchange capacity from acetylation or methylation. However, it cannot explain the fact that loss of exchange capacity is very much less than equivalent to the gain in acetyl and methoxyl content unless we simply assume that certain OH groups are not active in the base exchange reaction. Furthermore, it does not account for the presence of acetyl groups or nitrogen. However, it does show a low methoxyl and carbonyl content, and possesses groups which should give breaks in a potentiometric curve.

Siedlietzki,(53) by means of spectrographic analysis, has shown that humic acids from peats and other soils have a similar atomic arrangement, the central nucleus resembling that of lignin.

None of the above formulas shows the presence of nitrogen, an element whose presence in the pigment fraction was originally thought to be an impurity. However, it was not long before nitrogen was recognized as an essential part of the complex.

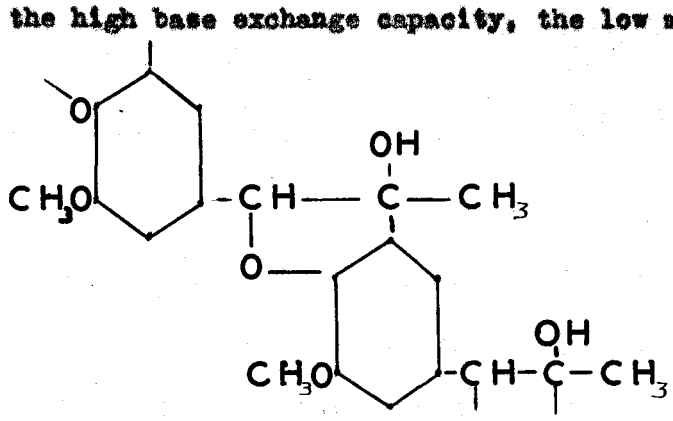
Andre[3] found that only a part of the nitrogen was removed by boiling humic acid with NaOH. Suzuki[56] concluded that nitrogen was not present as amino compounds but as a kind of protein, connected with the black substance. Fuchs[14] found that ammonia reacted with humic acid to form a compound from which only a part of the nitrogen can be removed by distilling with alkali, and he argued that the oxygen of a furan ring had been replaced by nitrogen, forming a pyrrole ring. This compound was found to be very resistant to oxidation.

Shmook[52] stated that the humic acid fraction was a complex combination of a soil protein molecule with the characteristic nitrogen free group, lignins or their transformation products. Similar conclusions were reached by Hobson & Page[18] who stated that nitrogen was present in humic acid as a polypeptide. They considered humic acid, therefore, to consist of a non-nitrogenous part and a protein, linked in a more intimate manner than that of a colloidal complex formed by mutual precipitation.

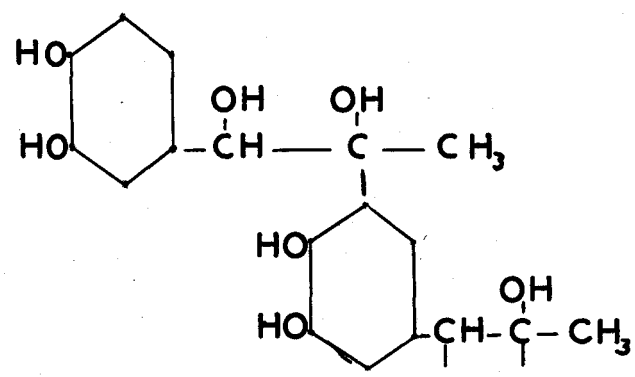
The presence of nitrogen has thus been explained either by assuming that it had replaced oxygen in a cyclic compound, or by the more generally accepted theory, that it is a protein-like molecule bound to a nitrogen-free compound.

The above structural formulas then, might be viewed as répresenting the non-nitrogenous part of the complex. Since many of the characteristics of the black pigment are similar to those of soil lignin, it was concluded

that the non-nitrogenous portion of the soil black pigment consisted of a modified lignin unit. Freudenberg[12] suggested a formula for lignin having the following structural unit. This formula could not account for the high base exchange capacity, the low methoxyl content, or the ability for acetylation and methylation. It also assumes the homogeneity of lignin which has never been proved.

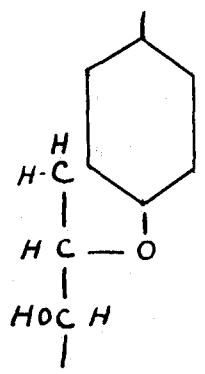



Decomposition in the soil, of the above formula, might result in the following structural unit.

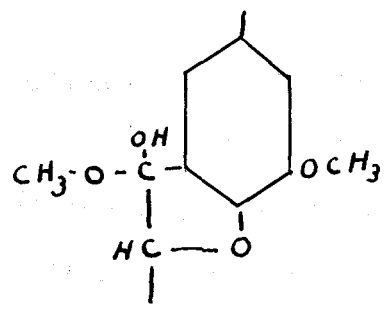



The phenolic hydroxyl content of this unit would readily account for the high base exchange capacity of the pigment. The secondary and tertiary alcohol groups would account for the fact that gain in acetyl and methoxyl content is much greater than the corresponding decrease in base exchange capacity, since these groups would not be involved in the base exchange reaction.

The heterogeneity of lignin was recognized in a recent paper by Freudenberg[13] who visualized the constitution of lignin as follows:



69%



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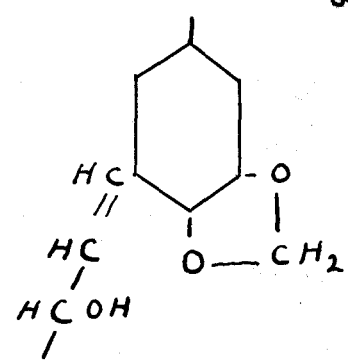
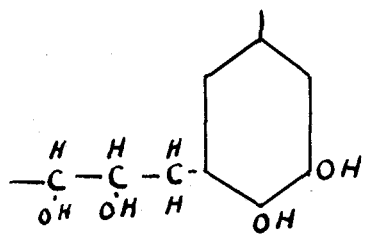
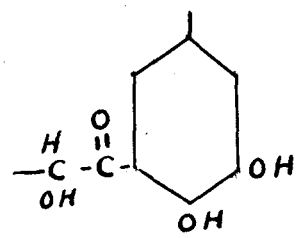
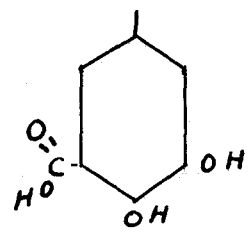


9%

These structural units account for all the properties of lignin, but in no way account for all of those of the black pigment. However, since further degradation of lignin must take place within the soil we might assume that hydrolysis of the methoxyl groups and fissure of the cyclic portion containing oxygen is the first step. This would result in units having the following structure:







These units readily account for the low methoxyl, acetyl, and carbonyl content of the soil black pigment. Furthermore, the phenolic hydroxyls easily explain the high base exchange capacity and the loss of exchange capacity due to methylation and acetylation. The secondary alcohol groups offer an explanation for the fact that gain in methoxyl and acetyl content is much greater than the loss of base exchange capacity. Furthermore, the presence of a carboxyl group would explain the break in the potentiometric curves at a relatively low pH. Finally, the fact that plant lignin itself

is not homogeneous would account for the slight differences found in the pigments extracted from widely separated soils.

## GEOGRAPHIC DISTRIBUTION OF THE BLACK PIGMENT

### Introduction:

The transformation of animal and plant residues into the soil black pigment fraction, and the weathering of rocks, constitute two of the most important processes in the development of soils. These two processes form substances which interact, thus causing the formation of new compounds. Soil formation then, is due not only to decomposition and disintegration but also to synthesis and accumulation. Furthermore, the organic matter content of any soil is intimately associated with the history of that soil; the importance of this fraction in characterizing the soil, and the essential part it plays in soil processes are well recognized. The formation of soil organic matter and likewise of soil pigment is a function of such factors as climate, vegetation, biological activities, parent material, topography, and drainage.

Since we do not know which of the above mentioned factors exert the greatest influence upon pigment formation, it will be necessary to limit the study to those factors for which we have definite values. The other factors, we must assume remain constant. Of these six factors, climate, vegetation, and biological activities are probably the most important. Our data concerning the microbiological activity in the various soil regions is very meager. The influence of vegetation can be noted in a very general manner, but practically all the soils studied in this investigation are from a prairie region. On the other hand, we have extensive data dealing with the climate of various areas.

From the point of view of soil formation, the important climatic factors are temperature, precipitation, and evaporation. The latter two are generally combined into a single quotient called the humidity factor. Therefore, we can say that the pigment content of a soil is a function of the climate.

$$\text{Pigment content} = f(\text{climate}) \quad (20)$$ or

$$\text{Pigment content} = f(\text{temperature, humidity})$$

The correlation of black pigment and climate has therefore been limited to the influence of temperature and of the humidity factor, N. S. Quotient.

## Temperature

Although it has been recognized for several decades that the nature of the climate of a certain region has a very important effect upon the type of soil developed there, the effect of temperature upon organic matter formation has not been very thoroughly studied. Mohr,[37] from his observations in the tropics (Java and Sumatra) stated that humus could not exist in a well aerated soil of the moist warm tropics where an average temperature of 25°C prevails.

From Vageler's text on tropical soils,[58] one concludes that the soil black pigment is practically missing from these soils, although the organic matter content may be quite high under certain conditions.

Jenny[21] found that in high altitudes where the annual temperature was around 0°C the soils contained as much as 20-40 per cent organic matter.

## Humidity Factors

Lang[23] in 1915 gave the first quantitative expression to the relation between soil and climate. He assumed that variations in temperature and

evaporation are usually directly related. Therefore, he combined precipitation and temperature in his "rain factors," which were calculated by dividing the rainfall (in millimeters) by the mean annual temperature (centigrade) of the specific region.

Meyer,[58] from his observations in Europe came to the conclusion that, next to precipitation, the saturation deficit was the most important factor as a measure of evaporation. He applied the term "N. S. Quotients" (Niederschlag and Sattigungsdefizit) to the ratio of the annual precipitation in millimeters to the absolute saturation deficit of the air.

Historical:

A review of the literature indicated that very little work had been attempted on the problem of the geographic distribution of soil black pigment. The work that has been published deals with the distribution of nitrogen or organic matter. These two, of course, are very closely associated since they bear a fairly constant ratio to each other. However, it does not necessarily follow that organic matter content and pigment content of a soil have any such close relationship.

Some authors hold that in red soils, or soils in subtropic and tropical regions, no such black pigment is to be found, while other investigators maintain that the black pigment is present but its presence is masked by various hydrated oxides.

In 1916, Alway and Blish[2] studied the distribution of humus and humus-nitrogen in the state of Nebraska. They found that the "soluble pigment" (that portion of the organic matter soluble in dilute ammonium hydroxide) in the surface foot, decreased in passing from east to west across the state. They also concluded that soil color agreed in general with the

amounts of "soluble pigment" found by the colorimetric method.

Brown and O'Neal[6] likewise found that with an increase in humus content the soil color changed from gray to dark brown to black, for those soils that had developed on loess.

Collection of soil samples:

In grassland soils it has been found that organic matter and nitrogen content decrease regularly with depth[47] and apparently independently of horizonation. Furthermore, the greater portion of soil organic matter is confined to the surface soil. Therefore, in this investigation, samples were taken to an empirical depth, and not by horizons. Only the surface six inches of soil were sampled. However, in a few cases the second six inches were also secured. Over three hundred virgin samples were collected from meadows and clean roadsides in various counties and states. The samples consisted of composites of from three to ten cores taken in a straight line over a uniform portion of the field being sampled. Sampling was accomplished by means of a tube and spade. Before pressing the tube or spade into the soil any vegetation or debris on the surface was cut away.

The location of all samples is shown in Figures 11 and 12 and again in Tables 1, 2, 3, and 4 in the appendix.

Preparation of samples:

The samples first of all were crushed and passed through a screen having holes 1/8 inch in diameter. The stones that collected on the screen were discarded but the roots, rhizomes, et cetera, that collected were chopped up and returned to the sample. The soils were then air dried, and ground fine enough to pass through a sieve having holes 1 mm. in

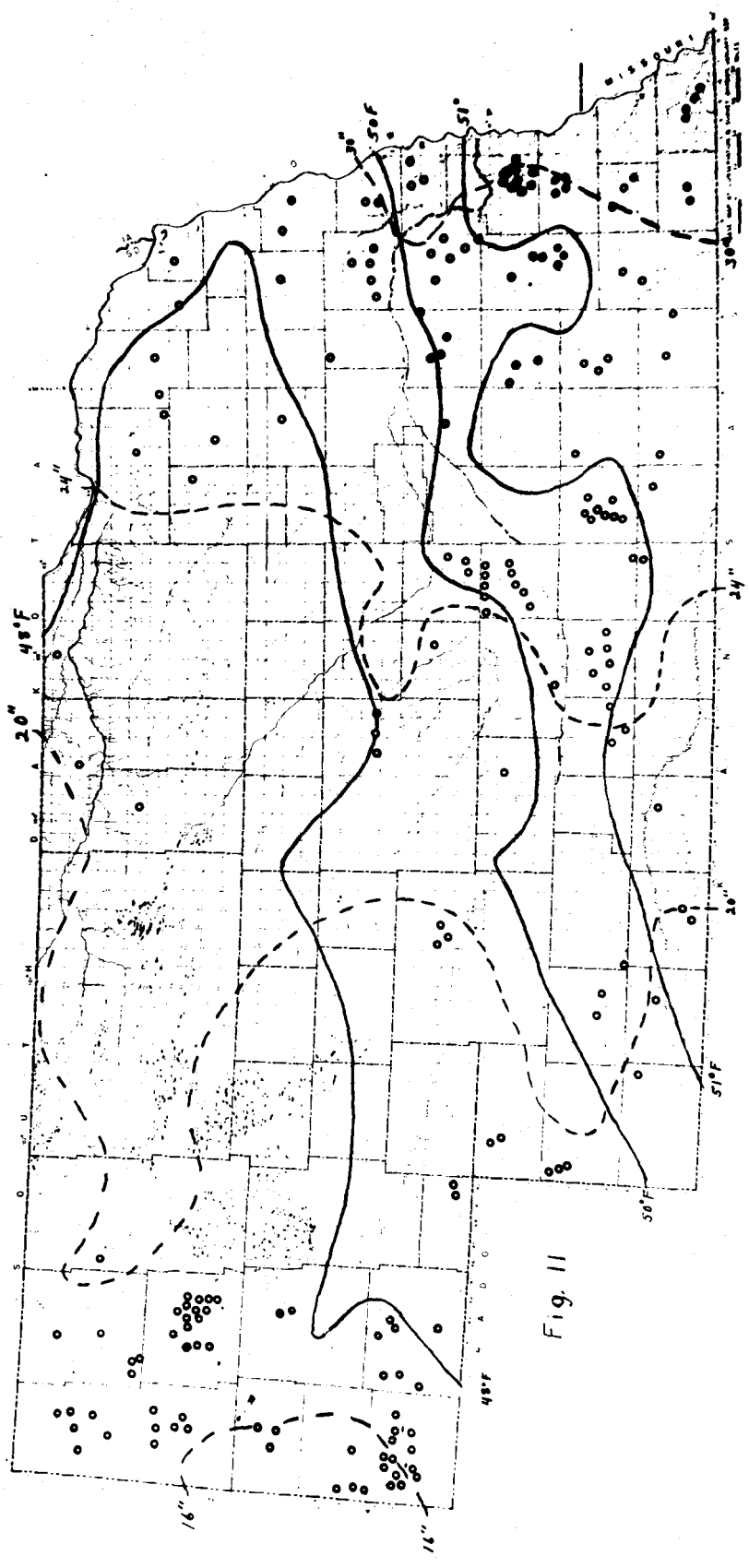
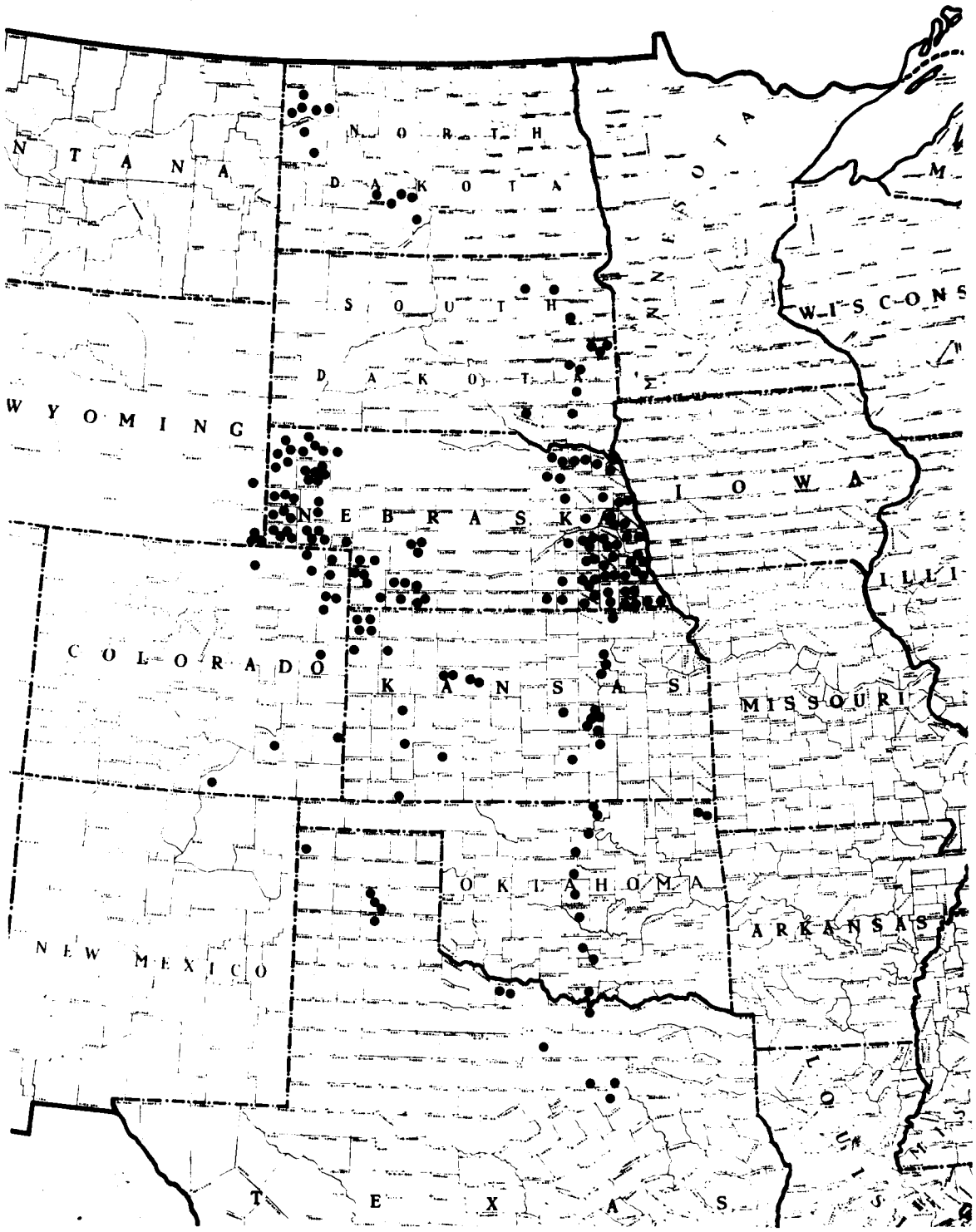



Fig. 11

FIG. 12


NORTH DAKOTA
SOUTH DAKOTA
WYOMING
NEBRASKA
COLORADO
KANSAS
OKLAHOMA
NEW MEXICO
TEXAS
IOWA
MISSOURI
ARKANSAS
WISCONSIN
ILLINOIS

The hygroscopic moisture, hygroscopic coefficient, and per cent organic matter were determined on every sample.

Colorimetric Determination of pigment content

Alway[2] concluded that the colorimetric determination of ammonia-soluble organic matter was fairly satisfactory for surface soils. Oden, Stadnikoff, Mehl and others reported the method as satisfactory for determining the amount of humus.[60]

Carr[7] found no relationship between the amount of humus and degree of color of the ammonia-soluble matter. He stated, "The color is probably due to the presence of pigments . . . . and is not related to humus content."

Eden[9] concluded the colorimetric determination of humic matter was sound in principle.

Strzemienska[55] extracted soils high in organic matter content with N/2 sodium carbonate solutions, and compared the extracts in a colorimeter. He concluded that there was a constant proportion between a "black substance" and lignin-humic complex.

However, the colorimetric determination of humus has been subjected to considerable criticism. Probably the most logical explanation for its limitations was pointed out by Gortner,[16] who illustrated that there are four variables which influence the color of the humus solution. These are the amount of black pigment present, the proportion of practically colorless compounds, the presence of a red-brown coloring material extracted from dead plant materials, and the amount and nature of the vegetable indicators.

Thus no reliance should be placed in this method for the absolute determination of humus content of a soil. However, if the standard humus

solution used is prepared from a soil of the same locality as those from which the humus extracts are to be measured, then the method is fairly reliable.(1)

Except for verifying this latter statement, no attempt was made to calculate the absolute amount of humus present in the soil samples by the colorimetric method. Instead, the relative per cent humus was determined gravimetrically.

Still another objection raised was the fact that the black solutions in strong alkali rapidly lost color. However, no such loss of color took place in the 4 per cent ammoniacal suspensions. Several extracts were left in glass bottles and placed near windows where they were subjected to direct sunlight for a certain period each day. They were allowed to stand for twelve months and were read at intervals in the colorimeter. The loss in intensity of color is shown below:

| Sample | Relative Humus Color | Time Exposed |
|---|---|---|
| 1 | 87 | 0 |
| 2 | 45 | 0 |
| 3 | 60 | 0 |
| 1 | 83 | 4 weeks |
| 2 | 40 | 4 weeks |
| 3 | 54 | 4 weeks |
| 1 | 35 | 55 weeks |
| 2 | 34 | 55 weeks |
| 3 | 48 | 55 weeks |

In four weeks' time there has been practically no loss of color. Furthermore, during this investigation the suspensions were read within a few hours after their extraction, consequently no serious error was introduced.

In general, soil organic matter may be considered as consisting

principally of two fractions. One portion consists of colorless or very slightly colored substances, while the other fraction is highly colored. Therefore, if the ammoniacal extracts of various soil samples were diluted until they contained equal amounts of humus, as determined gravimetrically, and were then read colorimetrically against some color standard, one could obtain a measure of the relative humus color and the relative pigment content of the different extracts.

Thus the relative humus color of the various extracts is simply the ratio of:

$$\frac{\text{standard reading} \times 100}{\text{reading of extract being examined}}$$

whereas, the relative pigment content was obtained by multiplying the relative humus color by

$$\frac{\text{per cent humus in the extract being studied}}{\text{per cent humus in the standard}}$$

This affords a relative method for studying the distribution of the black pigment.

The colorimeter used was a Campbell-Hurley. It consists primarily of two cylinders, one of which contains a column of liquid at a definite level, while the level of the standard solution in the other cylinder is varied at will by a mechanical method. For illumination, north daylight was used.

The chief source of error has been mentioned previously, namely, that the color produced by the dissolved black pigment is not the only color present in the solutions. A pale yellow color, from dead plant residues, and the presence of organic indicators, will affect the reading. Besides this, there are, of course, the typical sources of error encountered in colorimetric work, such as mechanical errors of the colorimeter, errors arising from varied light, errors in readings, errors of dilution, and

errors arising from turbidity of some of the solutions. These latter errors, however, were minimized as much as possible and are therefore relatively unimportant.

Preparation of the Standard Solution

The standard solution used in all colorimetric determinations was obtained from a Barnes silt loam soil of McCook county, South Dakota. This soil had an organic matter content of 5.22 per cent, a relative humus content of 1.88 per cent, and 3.9 per cent hygroscopic moisture. Fifty grams of the oven dry soil were mixed with an equal weight of clean quartz sand and placed in a percolation tube. It was then leached with 1 per cent hydrochloric acid until no test for calcium could be obtained in the leachate. Following this, distilled water saturated with carbon dioxide was passed through the soil column until no test for chloride was obtained in the leachate. Finally a solution consisting of 4 per cent ammonia and 2 per cent ammonium carbonate was percolated through the soil and exactly 1200 cubic centimeters of the jet black solution was collected. Fifty cubic centimeters of the extract contained 0.0392 gram of ash-free humus, which is equivalent to 1.88 per cent. Two hundred cubic centimeters of the extract was then diluted with 4 per cent ammonia to 1568 cubic centimeters reducing the humus content to 100 parts per million, or one part of humus to 10,000 parts of solution. This was the standard solution, containing 100 p. p. m. of humus, that was used in the colorimeter. Consequently, the humus content of any extract, in parts per million of solution, in parts per million of soil, or the grams of humus leached out of the soil sample could be readily calculated. For example:

$$\text{Humus content in p.p.m. of solution} = \frac{(a) \times (b) \times 100}{(x)}$$

$$\text{Grams humus leached from the soil sample} = \frac{(a)x(b)x(c)x\ .0001}{(x)}$$

where (a) = reading of the standard solution in colorimeter

(b) = dilution in cubic centimeters of the extract being examined

(c) = volume of extract in cubic centimeters

(x) = reading of the colorimeter cylinder containing the extract being studied.

(.0001 grams = weight of humus in 1 cc of the color standard)

Method of Extraction

The equivalent of 50 grams of oven dry soil of each sample was weighed out and thoroughly mixed with an equal weight of clean quartz sand. The mixture was placed in a percolation tube,[49] and leached with one per cent hydrochloric acid until the leachate was free of calcium. As a rule this required from 400 to 600 cc depending on the sample. The acid leachate in all cases was pale yellow in color, except for the forest soils where it acquired a more reddish tint. Each sample was run in duplicate. The sample was next leached with distilled water saturated with carbon dioxide until the leachate was free of chloride. Finally, a solution consisting of 4 per cent ammonia and 2 per cent ammonium carbonate was percolated through the soil column and exactly 1200 cc of the jet black extract collected. The ammonia solution was delivered to the percolating tube through ground glass tips calibrated to deliver 1.5 cc per minute. Thus, each sample was in contact with the extracting solution for approximately the same length of time.

The relative per cent of humus extracted and its ash content were determined gravimetrically for every sample. For a large number of samples the humus content was also determined colorimetrically. Finally, an aliquot of each ammoniacal extract was withdrawn, diluted until

it contained 100 parts of humus per million of solution and was compared in the colorimeter against the standard. The relative humus color and pigment content were calculated from these readings.

The extracts from several of the samples were collected in 300 cc portions, aliquots withdrawn, diluted, and read in the colorimeter. Parts of humus per million of solution were calculated and plotted against volume of the extract in cubic centimeters. Five samples chosen at random are shown in Fig. 10.

These curves showing parts per million of humus plotted against volume of extract in a general way fall about as one would surmise. The first 300 cc portions of the ammoniacal extracts from the Kansas samples have a much higher content of humus than do the samples from southern Oklahoma. It therefore requires a slightly larger volume of solution before the extracts from the more northern soils become colorless or very faintly colored.

The ammoniacal leachate of practically every sample was very nearly colorless after 600 cc of solution had percolated through the soil column. This likewise is illustrated by the curves in Fig. 10. Consequently, with many samples only 800 cc of solution was percolated through the tube.

After being leached with dilute acid, every calcareous soil encountered, plugged the percolation tube when an attempt was made to leach the soil column with carbon dioxide-saturated water. It would be interesting to determine the cause of this phenomenon.

Organic Matter and Humus Determination

The organic matter content of all samples was determined by the hydrogen peroxide method. All samples were run in duplicate and made to check within 0.2 per cent. Most of them checked within 0.1 of one per cent.

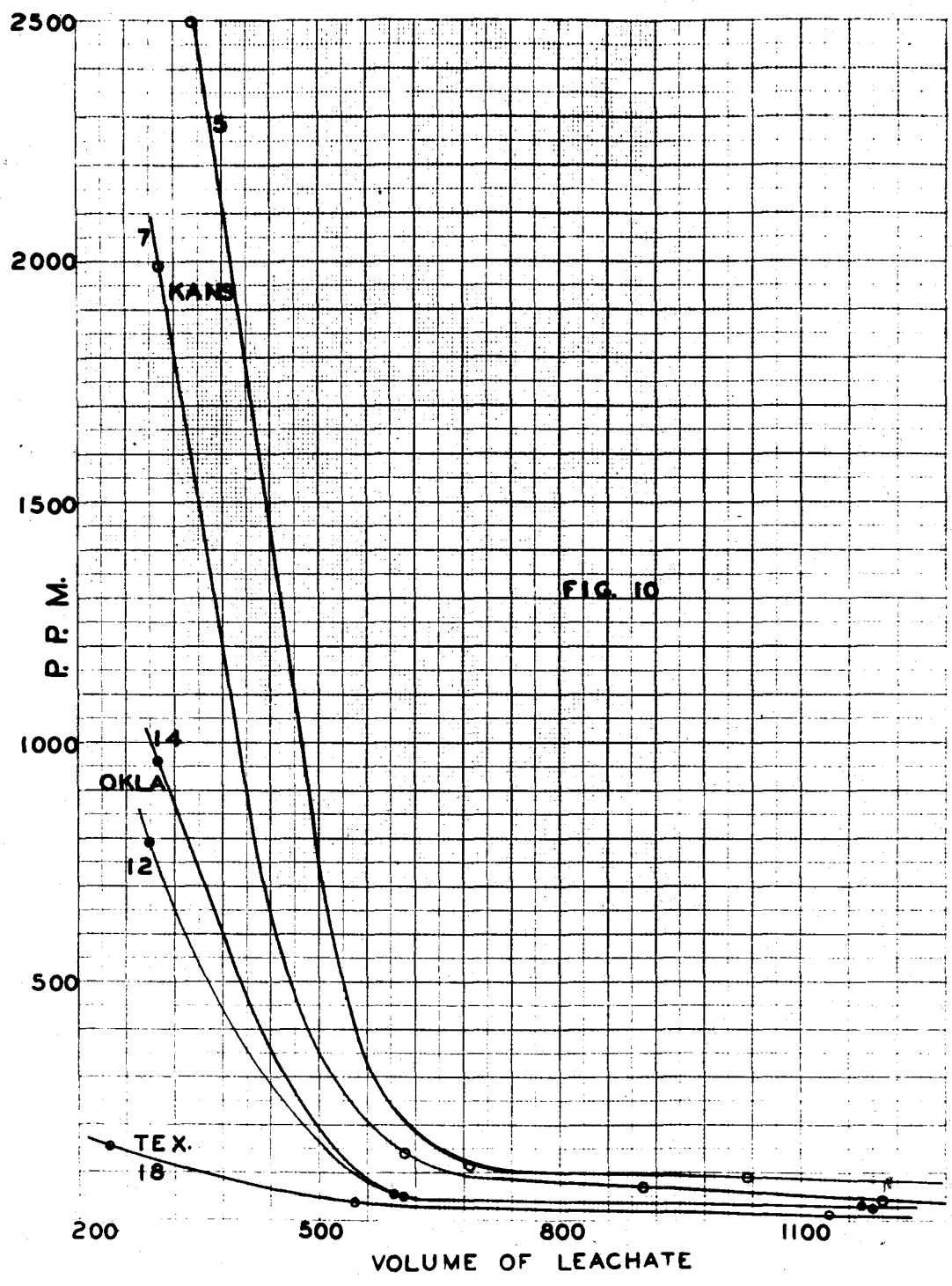

2500
2000
1500
1000
500
P. M.
9
5
7
KANS
14
OKLA
12
TEX.
18
200
500
800
1100
VOLUME OF LEACHATE

FIG. 10

Samples having a high content of carbonate were treated with dilute acid to remove the carbonate, and the organic matter was determined on the portion remaining.

Relative humus content was determined by evaporating an aliquot of the ammoniacal extract to dryness in a platinum crucible and weighing. The sample was then ignited, and weighed again. From this difference in weight relative per cent humus was calculated.

Incubations

Can the relative per cent humus, pigment content, or humus color be increased by the addition of organic matter to a soil or sand medium? In an attempt to answer this question the following experiment was performed.

The equivalent of fifteen grams of over-dry organic matter and 285 grams of oven-dry Rosebud silt loam soil were placed in each of seven pint milk bottles and thoroughly shaken. Sufficient distilled water was then added to bring the water content up to the moisture equivalent of the soil. The bottles were then capped with cheese cloth, weighed, and placed in a constant temperature room. The weight of each bottle was kept constant by frequent additions of distilled water.

Three organic materials were used in a soil medium, sand medium, and in a sand medium inoculated with water leached through a Marshall surface soil. The organic materials consisted of:

| | Per cent N | Per cent $H_2O$ |
|---|---|---|
| wheat straw | 0.42 | 7.4 |
| horse manure | 1.35 | 7.5 |
| alfalfa tops | 2.69 | 7.9 |

These composts were sampled at intervals of four, eight, sixteen, thirty-two, et cetera, weeks. Per cent moisture was determined on each

sample and the equivalent of fifty grams of oven-dry material was placed in percolation tubes and leached exactly as the soil samples were leached. Per cent humus was determined gravimetrically, and the extracts were compared against the same standard in the colorimeter. The results are given in Table 6.

TABLE 6

Effect of Wheat Straw on Pigment Formation

| Medium | Sample Number | Per cent Humus | Relative Humus Color | Relative Pigment Content | Time Interval Between Samplings |
|---|---|---|---|---|---|
| Rosebud Soil | X | 0.78 | 87 | 36 | |
| | 1 | 0.99 | 75 | 41 | 2 wks. |
| | 2 | 1.10 | 73 | 42 | 4 " |
| | 3 | 0.96 | 75 | 45 | 8 " |
| | 4 | 1.11 | 64 | 39 | 16 " |
| | 5 | ---- | 74 | 41 | 32 " |
| | 6 | ---- | 51 | 30 | 38 " |
| | 7 | 0.87 | 39 | 18 | 60 " |
| | | HORSE MANURE | | | |
| Rosebud Soil | 10 | 1.14 | 70 | 45 | 2 " |
| | 11 | 1.25 | 67 | 44 | 4 " |
| | 12 | 1.26 | 59 | 50 | 8 " |
| | 13 | 1.11 | 63 | 37 | 16 " |
| | 14 | ---- | 65 | 42 | 32 " |
| | 15 | ---- | 60 | 38 | 38 " |
| | 16 | 0.96 | 39 | 20 | 60 " |
| | | ALFALFA TOPS | | | |
| Rosebud Soil | 20 | 1.00 | 72 | 41 | 2 " |
| | 21 | 1.17 | 71 | 44 | 4 " |
| | 22 | 1.27 | 62 | 42 | 8 " |
| | 23 | 1.17 | 64 | 40 | 16 " |
| | 24 | ---- | 62 | 38 | 32 " |
| | 25 | ---- | 47 | 30 | 38 " |
| | 26 | 0.95 | 40 | 20 | 60 " |

| | Sample Number | Per cent Humus | Relative Humus Color | Relative Pigment Content | Time Interval Between Samplings |
|---|---|---|---|---|---|
| | | | WHEAT STRAW | | |
| Sand | 100 | 0.32 | | | 0 |
| | 101 | 0.34 | The solutions were too greenish yellow to match the standard | | 2 wks. |
| | 102 | 0.30 | | | 4 " |
| | 103 | 0.37 | | | 8 " |
| | 104 | 0.31 | | | 16 " |
| | 105 | ---- | | | |
| | 106 | 0.39 | | | 60 " |
| | | | HORSE MANURE | | |
| Sand | 200 | 0.59 | | | 0 |
| | 201 | 0.44 | | | 2 " |
| | 202 | 0.50 | Too orange to match standard | | 4 " |
| | 203 | 0.61 | | | 8 " |
| | 204 | 0.41 | | | 16 " |
| | 205 | ---- | | | |
| | 206 | 0.66 | | | 60 " |
| | | | ALFALFA TOPS | | |
| Sand | 300 | 0.63 | | | 0 |
| | 301 | 0.38 | | | 2 " |
| | 302 | 0.39 | | | 4 " |
| | 303 | 0.46 | Too much greenish yellow color to match standard | | 8 " |
| | 304 | 0.42 | | | 16 " |
| | 305 | ---- | | | |
| | 306 | ---- | | | 60 " |

| Sample Number | Per cent Humus | Relative Humus Color | Relative Pigment Content | Time Interval Between Samplings |
|---|---|---|---|---|
| | | WHEAT STRAW | | |
| Sand (Inoculated | 110 | 0.33 | | 4 wks. |
| | 110' | 0.43 | Solutions were too green to match the standard. | 8 " |
| | 111 | | | |
| | 111' | 0.48 | | 55 " |
| | | HORSE MANURE | | |
| Sand (Inoculated) | 210 | 0.40 | | 4 wks. |
| | 210' | 0.36 | Solutions were too orange to afford a good match with the color standard. | 8 " |
| | 211 | | | |
| | 211' | 0.61 | | 55 " |

Discussion

In a study involving comparisons between different soil types it is well recognized that the soils used should possess approximately the same texture. Furthermore, all samples should be from normally developed well-drained soils of the uplands. Consequently, no samples were used from soils developed on a terrace or first bottom. In selecting the soil samples it was not expedient to obtain samples of equivalent texture. Therefore, the hygroscopic coefficient was used as an expression of texture. All samples can then easily be placed on an equivalent basis, and consequently the influence of any factors, which ordinarily would be completely overshadowed by differences in texture, can be illustrated.

Each point in Figures 13 to 18 inclusive represents the average of many samples reduced to an equivalent textural basis of a hygroscopic coefficient of 10. All the curves are drawn so that the sum of the squares of the deviations of all points are at a minimum.

Pigment, Temperature Relationship

In order to study the variation of the soil black pigment with temperature, humidity factors must be kept constant. Thus, black pigment = $f(T)_H$. N. S. quotients were calculated where sufficient data were given for every weather station within the area. They ranged from 261 to 138 for the eastern portion of the area studied and from 192 to 123 for the western portion. The greatest variation of the entire region being 123 to 261. The area, therefore, is classed as a semi-arid region since the N. S. quotients closely approach the 125-250 range set by Jenny.(21)

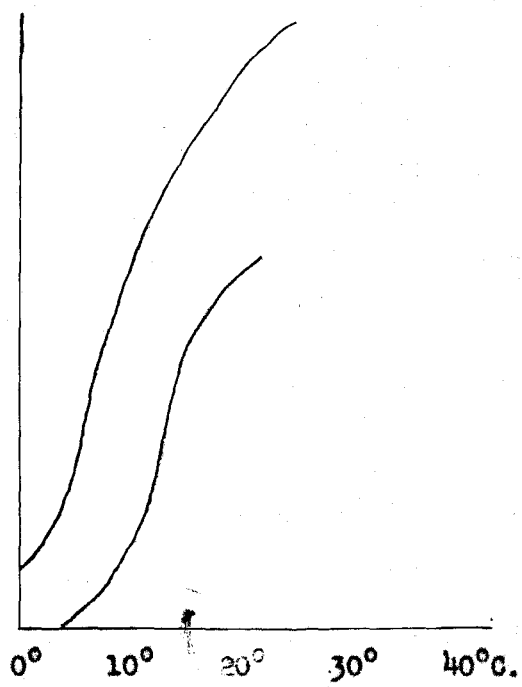
The samples listed in Table 1 (appendix) have been placed, as far as possible, from north to south along an isohyet line. Their location is illustrated in Fig. 12. The N. S. quotients along this eastern line range

from around 260 to 140. The data in Table 1A (appendix) are plotted in Figures 13 and 14.

The two curves in Fig. 13 are sigmoidal, and illustrate the distribution of soil organic matter and humus with changing temperature. As the temperature increases from around 43°F. to 55°F. both the per cent organic matter and humus decrease only slightly. However, from 55°F. to approximately 61°F. the loss of organic matter and soil humus is at a maximum. The curves represent a spread of 10°C. and illustrate the fact that for a fall of 10°C. in mean annual temperature the average soil organic matter and humus content increase about two times.

The shape of the organic matter curve is at variance with that of Jenny,[22] who found that the per cent soil organic matter in eastern United States decreases exponentially from north to south.

The curve (Fig. 13) in a general way appears to be complementary to the curve of Wollny, showing the effect of temperature on microbiological activity.



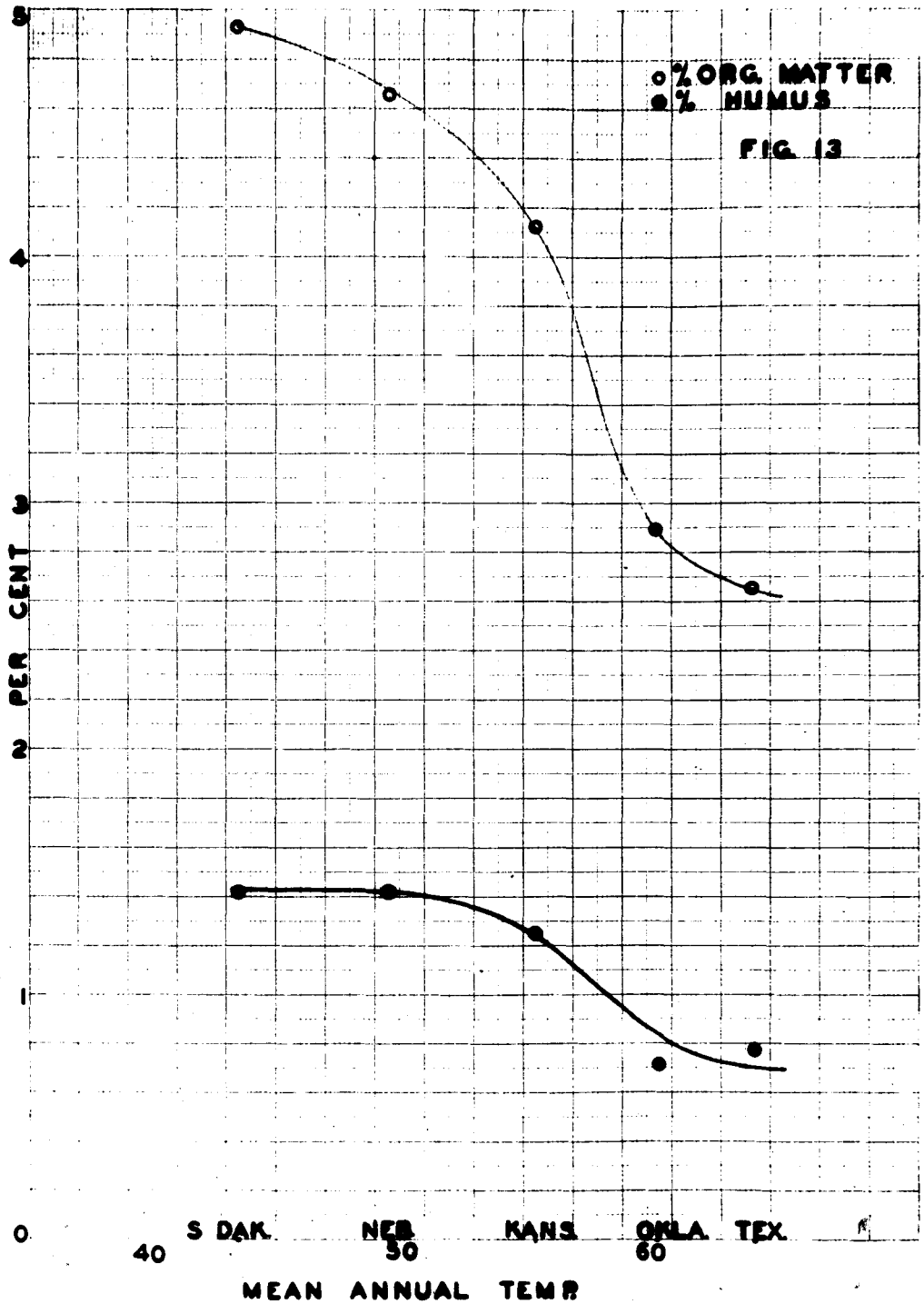

Effect of temperature on microbiological activity ($CO_2$- production) Wollny

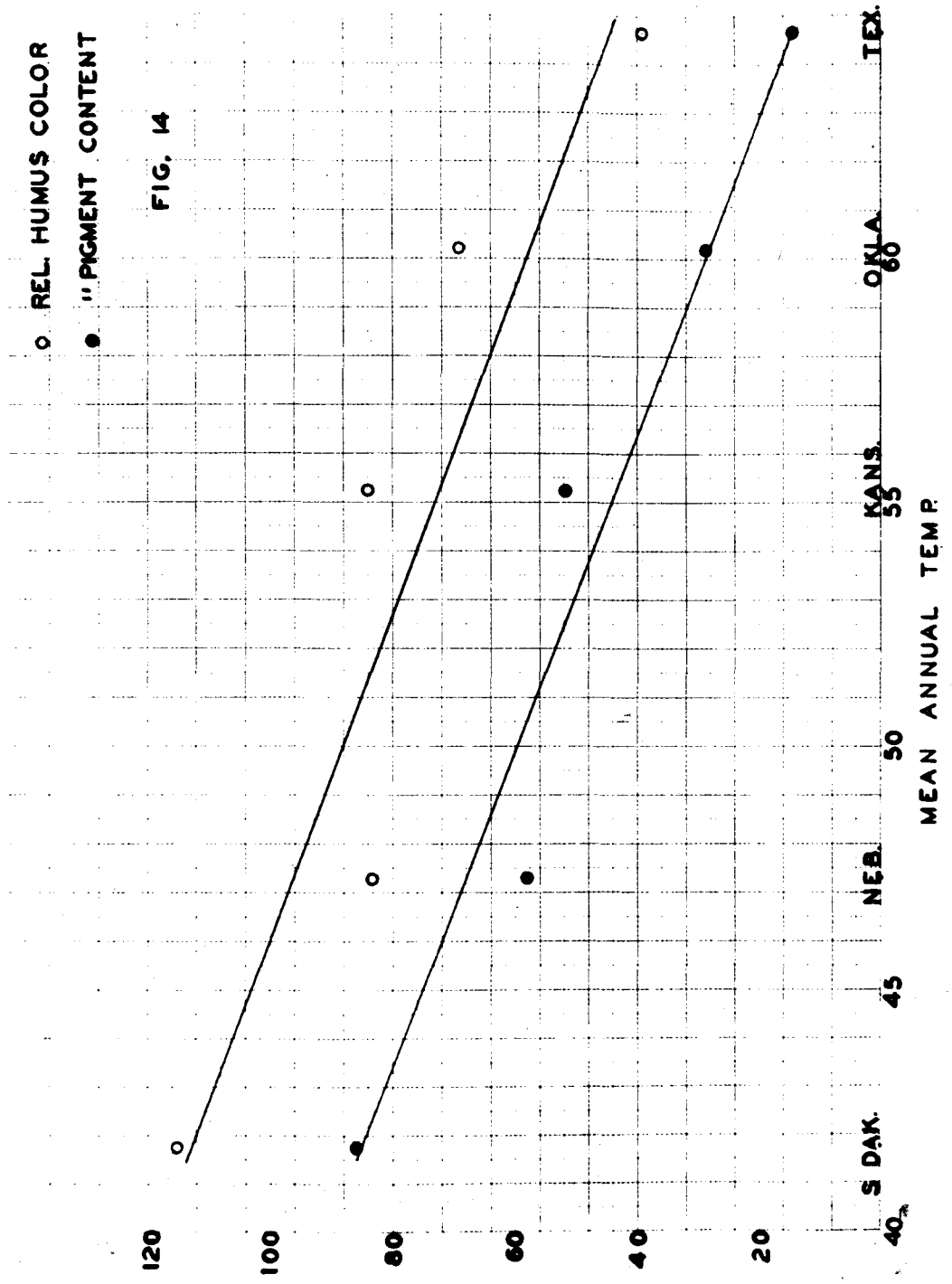
Fig. 14 brings out the linear relationship between both relative humus color, relative pigment content and temperature. Furthermore, for a 10°C. drop in mean annual temperature the relative pigment content of the soil has been increased five or six times, while the relative humus color has increased approximately three times.

The data in Table 2A (appendix) are plotted in Figures 15 and 16. These samples lie on an isohyet line to the west of those plotted in Figures 13 and 14, see Fig. 12. Consequently, we would expect somewhat lower organic matter, humus, and pigment contents than were obtained in the former series of samples. Again we note that the curve for per cent organic matter is sigmoidal. The points on the humus curve are possibly too widely scattered to allow any similar conclusion to be drawn.

In this area a drop of 10°C in mean annual temperature has increased the organic matter content only 1.5 times. This, and the fact that this curve is less precipitous than the one in Fig. 13, clearly illustrates that the influence of temperature is much greater in the region of higher rainfall. In other words, a slight difference in temperature results in a greater change in the organic matter content. The greatest change in organic matter content takes place between 48°F. and 54°F., across the panhandle of Nebraska, eastern Colorado and western Kansas.

Again in Fig. 16 the parallelism between humus color and pigment content is brought out. Similarly, there is a linear relationship between humus color, pigment content, and mean annual temperature. For a 10°C. drop in mean annual temperature the relative pigment content has increased two to two and one half times and the relative humus color about two to three times. The increase in relative pigment content is much less for this region than for that of the eastern area. This again shows the


o % ORG. MATTER
● % HUMUS
FIG. 13
PER CENT
5
4
3
2
1
0
S DAK.
NEB.
KANS.
OKLA.
TEX.
40
50
60
MEAN ANNUAL TEMP.


o REL. HUMUS COLOR
● " PIGMENT CONTENT
FIG. 14
120
100
80
60
40
20
S DAK.
NEB.
KANS.
OKLA.
TEX.
40
45
50
55
60
MEAN ANNUAL TEMP.

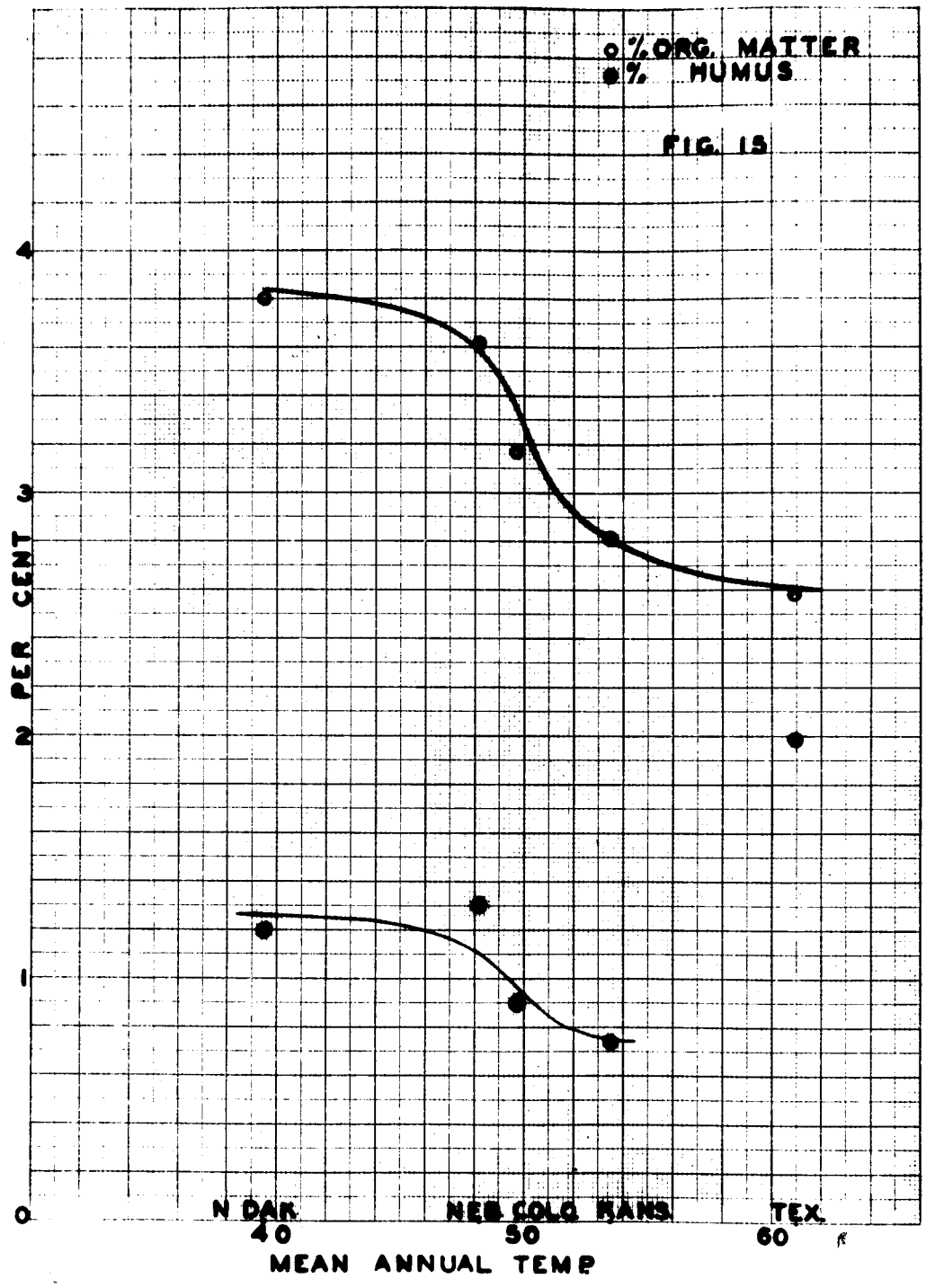
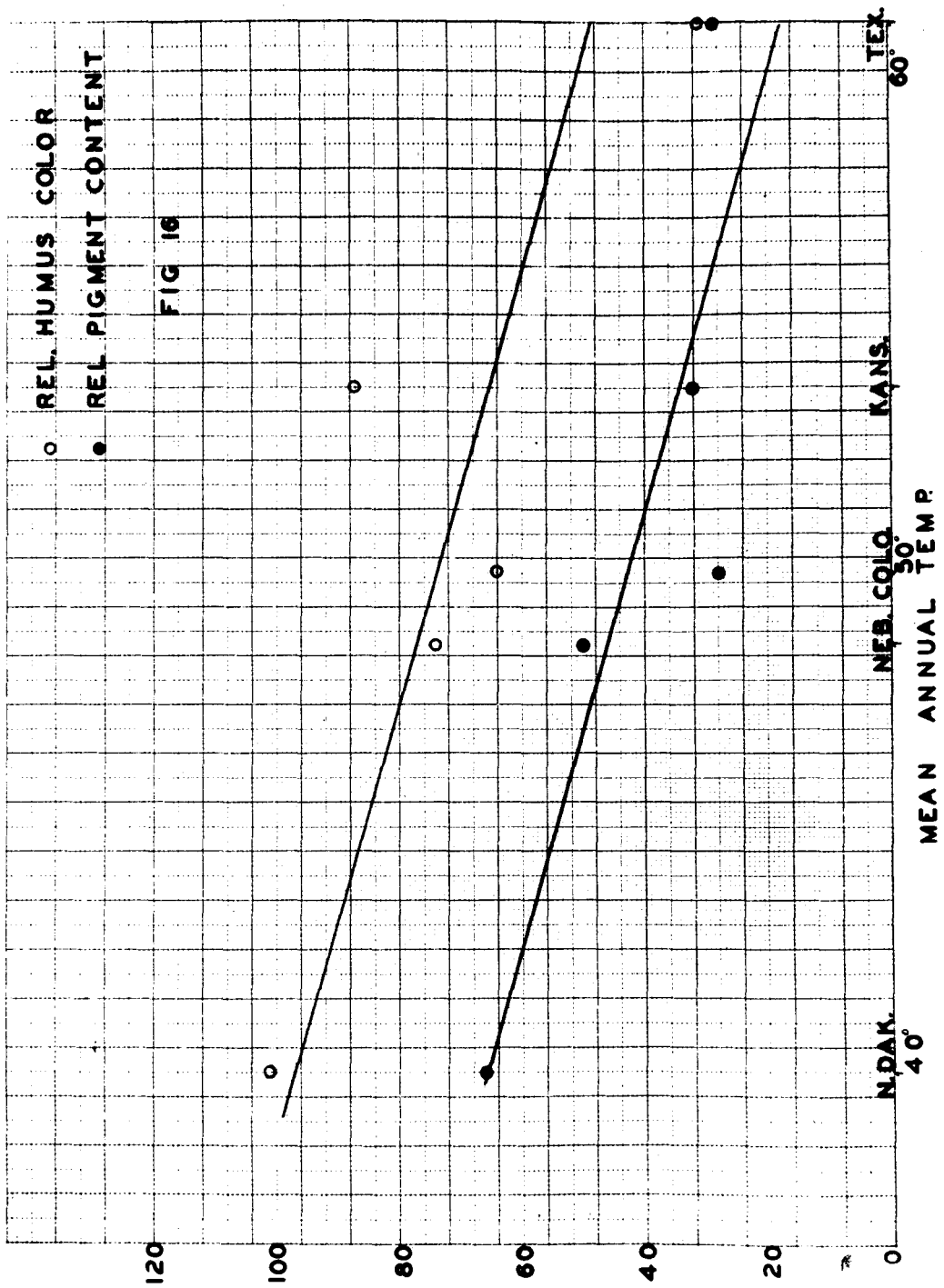

o % ORG. MATTER
● % HUMUS
PER CENT
4
2
1
0
N DAK
40
NEB COLO KANS
50
TEX.
60
MEAN ANNUAL TEMP
F

FIG. 15


o REL. HUMUS COLOR
• REL. PIGMENT CONTENT
FIG 16
120
100
80
60
40
20
0
N.DAK. 40°
NEB. COLO. 50°
KANS.
TEX. 60°
MEAN ANNUAL TEMP.

greater influence of temperature in the region of higher rainfall.

Relationship of Pigment and Organic Matter Content to Rainfall

The correlation between pigment content and rainfall was studied over a relatively small area. The soils investigated lie along an isotherm from eastern Nebraska into eastern Wyoming and Colorado. The location of the samples in Nebraska is primarily between the 48°F. and 50°F. isotherms, continuing on into Colorado and Wyoming, (Fig. 11). The samples were divided into three groups; group 1 consisted of those lying east of the 24" isohyetal line, group 2, those lying between the 20" and 24" lines, and group 3, composed of samples lying west of the 20" isohyet. In two instances a fourth group was added, consisting of samples in Wyoming and central Colorado.

The results are listed in Table 3 (appendix) and plotted in Figures 17 and 18. Groups 1 and 3 are very well represented by soil samples but group 2 contains a much smaller number. However, this group contains three samples which are composites of five fields each, and the data may therefore be more reliable than is apparent.

The curve showing the effect of precipitation on organic matter content, Fig. 17, apparently is just the converse of the curve obtained by Russel.(48) As the precipitation steadily increases, the effect on organic matter content becomes increasingly greater. The maximum effect is obtained between 22" and 27.5". Thus, a slight difference in precipitation in eastern Nebraska would have a much greater effect on the soil organic matter content than a corresponding difference of precipitation in western Nebraska.

The difference between the curve for organic matter content and the

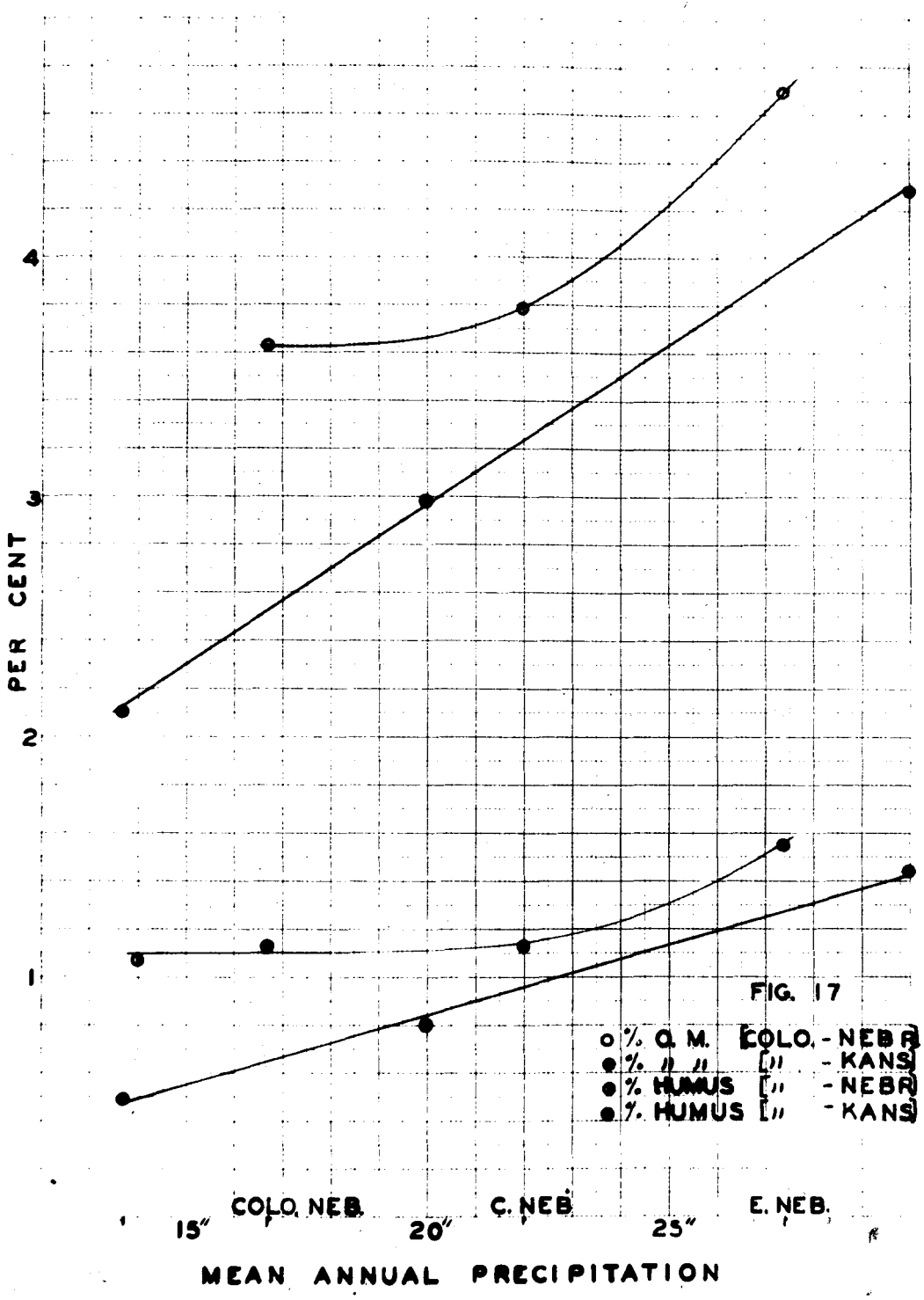

PER CENT
4
3
2
1
15"
20"
25"
COLO. NEB.
C. NEB.
E. NEB.
MEAN ANNUAL PRECIPITATION
o % O. M. [COLO. - NEBR.]
● % " " [" - KANS.]
● % HUMUS [" - NEBR.]
● % HUMUS [" - KANS.]

FIG. 17

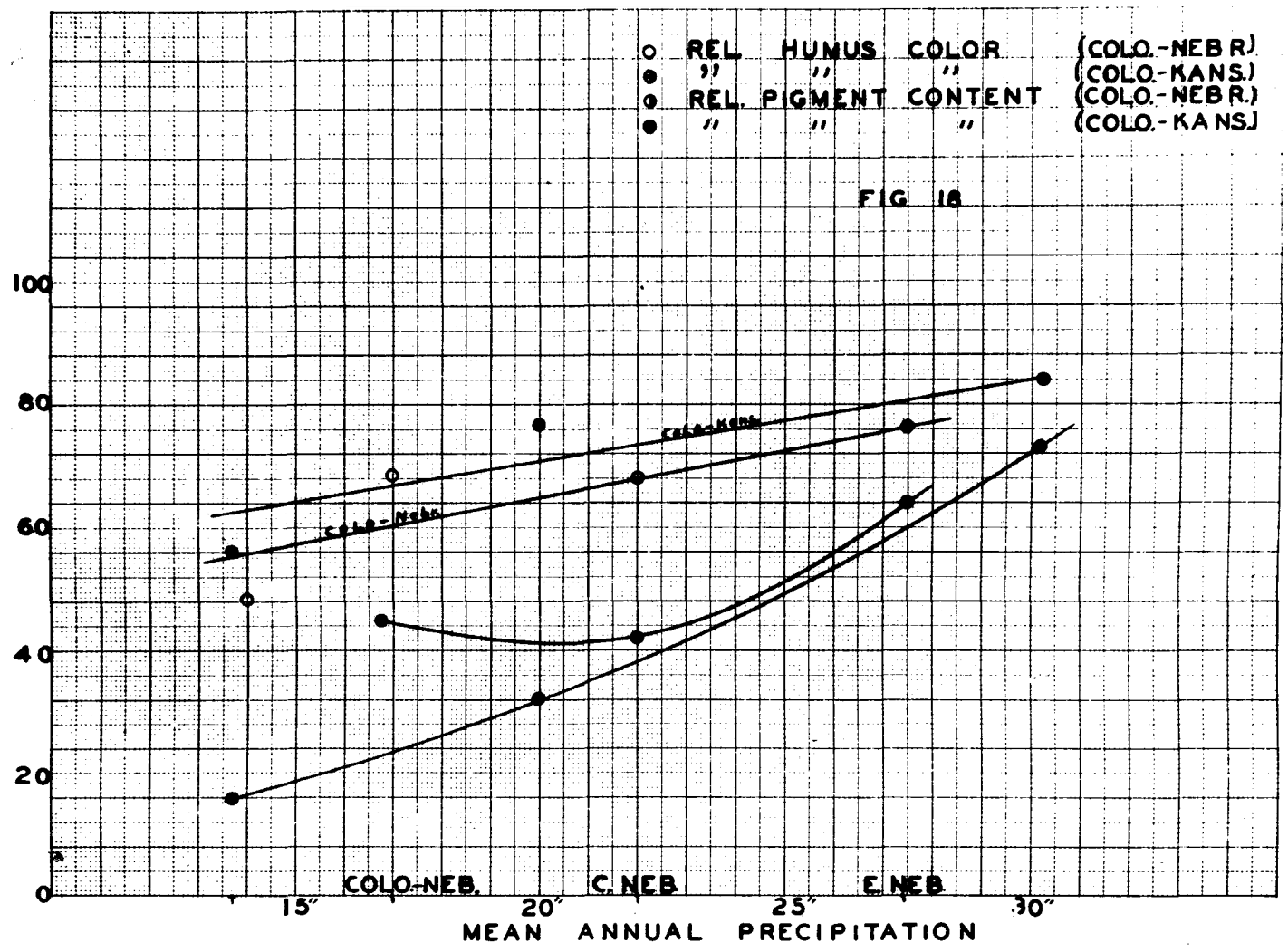
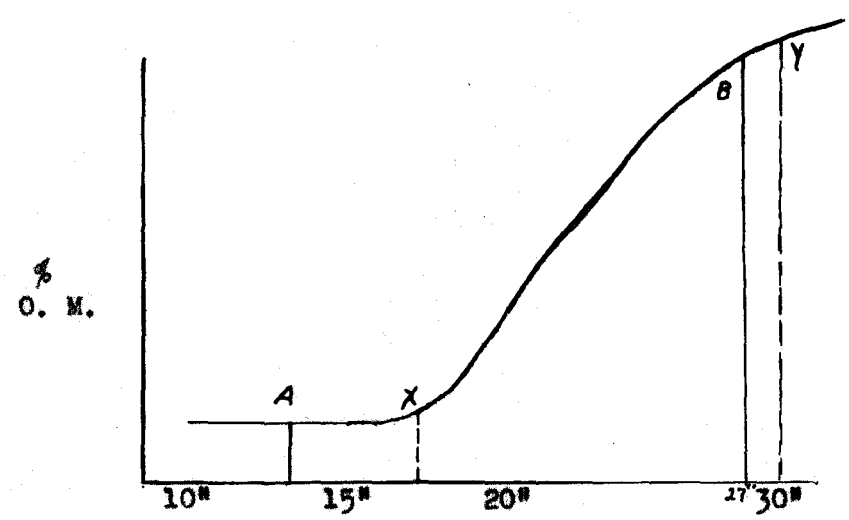

REL. HUMUS COLOR (COLO.-NEB R.)
" " " (COLO.-KANS.)
REL. PIGMENT CONTENT (COLO.-NEB R.)
" " " (COLO.-KANS.)
100
80
60
40
20
0
%
COLO-NEB.
C. NEB
E. NEB
15"
20"
25"
30"
MEAN ANNUAL PRECIPITATION

FIG 18

one obtained by Russel is not as great as it may appear. When the organic matter content of soils is plotted against mean annual precipitation one would expect to obtain a simple S-shaped curve, as indicated below:




The samples plotted by Russel lay between 16½" and 30", or between the points X and Y in the diagram. The curve in this portion has the same general shape as the one obtained by Russel. On the other hand, the samples in this investigation were situated between A and B. This portion of the curve agrees fairly well with the one obtained in Fig. 17. There are too few soil samples to establish the exact graphical nature of the relationship between organic matter content and rainfall, but apparently it is a curve.

However, the same type of curve is not obtained across eastern Colorado and western Kansas. Here there apparently is a linear relationship between organic matter content and precipitation. This discrepancy is possibly explained by the fact that for this latter curve only 3, 7, and

8 samples were averaged respectively, to obtain each datum from Colorado to east central Kansas.

The corresponding curves for relative humus content possess the same general characteristics as those above, (Fig. 17). As the precipitation gradually increases from 14" to 22" humus content of the soil remains practically constant. But a further increase of 5" causes a decided rise in humus content. Again, there apparently is a linear relationship between precipitation and humus content across Colorado and Kansas, just as there was with organic matter content.

It is quite evident from Fig. 18 that a linear relationship exists between relative humus color and precipitation. On the other hand, the curves for the relative pigment contents are very similar to the organic matter and humus curves across Nebraska, (Fig. 17). Even across Kansas this relationship is seen to be a curve.

The humus content of all soils in Nebraska and closely adjacent areas, when calculated by the colorimetric method, were found to agree rather closely with the humus content as obtained by the gravimetric method. However, for more southern or western soils the humus content as calculated colorimetrically was much too low, and conversely the humus content for more northern soils was too high. This discrepancy is due to the differences in relative pigment contents in the soils from the widely separated areas. As one travels southward the relative pigment content decreases and consequently the colorimetric readings will be too low. Farther north the relative pigment content increases and therefore the colorimetric reading will be too high.

Soils from the podsol, laterite, red and yellow, gray-brown forest,

prairie, chernozem, chestnut and brown groups have been leached and every one yielded a jet black to very dark brown solution. The extracts from the laterite, podsol, gray-brown forest, and red and yellow groups were a bit too orange to match the standard perfectly. This difference in color is probably significant and may indicate a difference in the constitution of the pigment not brought out by the reactions used in this investigation. Or it may merely indicate the presence of different indicators or materials in suspension.

With many of the samples studied it was found that although they might be relatively high in organic matter content it did not necessarily follow that they were the darkest colored soils or that they contained the most pigment. On the contrary, many soils relatively low in organic matter content had the highest relative amount of pigment.

Comparison of Soil Groups

From the data assembled in Table 5 a very general comparison of the relative pigment contents, per cent humus, relative humus color, and soil groups can be made.

TABLE 5

| Soil Group - Series | Per cent Humus | Per cent Organic Matter | Relative Humus Color | Relative Pigment Content | Hygroscopic Coeff. | Per cent Humus HCl10 | Relative Pigment Content HCl10 |
|---|---|---|---|---|---|---|---|
| Laterite - Nipe | 2.31 | --- | 38 | 46 | 19.0 | 1.21 | 24 |
| Red and Yellow | | | | | | | |
| Davidson | 1.23 | 4.20 | 20 | 13 | 14.0 | 0.88 | 9 |
| Cecil | 0.60 | 1.69 | 20 | 6 | 5.8 | 1.03 | 10 |
| Norfolk | 0.56 | 1.86 | 37 | 11 | 2.3 | 2.43 | 48 |
| Durham | 1.08 | 4.10 | 27 | 16 | 4.2 | 2.57 | 38 |
| Podsol - Onaway | 8.12 | --- | 48 | 208 | --- | ---- | -- |
| Gray-brown forest soils | | | | | | | |
| Ontonagon | 2.08 | 5.1 | 64 | 71 | 5.3 | 3.92 | 133 |
| - - - | 0.74 | 2.68 | 48 | 19 | 4.2 | 1.76 | 45 |
| Bellefontaine | 0.84 | 2.86 | 76 | 34 | 3.8 | 2.21 | 89 |
| Fox | 0.76 | 2.32 | 60 | 24 | 4.5 | 1.68 | 53 |
| Newton | 0.83 | 2.32 | 51 | 22 | 2.9 | 2.86 | 76 |
| - - - | 1.45 | --- | 62 | 48 | --- | ---- | -- |
| Chernozem | | | | | | | |
| Barnes | 1.77 | 5.88 | 137 | 128 | 15 | 1.18 | 86 |
| Barnes | 1.79 | 5.94 | 121 | 115 | 14.4 | 1.24 | 80 |
| Barnes | 2.55 | 6.97 | 138 | 187 | 15.5 | 1.64 | 121 |
| Barnes | 1.88 | 5.85 | 109 | 109 | 13.5 | 1.39 | 80 |

(Samples are from the top 6 - 8")

Concerning the relative humus content we note that the podsol possesses the greatest amount. Making our comparisons on an equivalent textural basis we note further that the gray-brown forest soils are second, followed by the red and yellow group, the chernozem group and finally the laterite. Curiously, the Nipe sample was found to contain practically as much humus as the Barnes soils.

The relative pigment content of these soil groups was found to be highest for the podsol, followed by the chernozem, gray-brown forest, red and yellow, and laterite groups. The high value for the podsol was no doubt due to the presence of a large amount of partially decomposed organic debris from the forest floor. Thus, if we consider the mineral portion of the surface soil, the chernozem group possesses the highest pigment content, the gray-brown forest group comes next in line, and finally the red and yellow and laterite groups. The soil groups fall in the same order when we consider the relative humus color of the extracts. The extracts from the chernozem soils were by far the blackest, as shown by column three. Next in order came the gray-brown forest soils, and the podsol. The Nipe extract was as dark as any of the extracts from soils of the red and yellow groups.

The formation of humus materials from stable manures is somewhat different from that of humus formation in soils. The material comprising the bulk of the manure has undergone digestion, and has thus been greatly modified from the original plant and animal substances. One would expect manure to undergo further decomposition quite rapidly when placed in a compost and kept under favorable conditions of moisture and aeration.

As the stable manure gradually decomposed the incubation samples,

number 200 to 206 inclusive, became much darker in color, but the per cent of ammonia-soluble material did not increase with age. The extracts, however, did become darker colored and towards the end gave a fair comparison with the standard.

The addition of the organic materials to the Rosebud soil did increase the per cent of ammonia-soluble material of the samples, slightly. Alfalfa and horse manure increased the per cent humus the most and straw the least. This is as one would expect, since plant materials containing a large amount of nitrogen decompose quite rapidly in the soil. However, the relative humus color and relative pigment content of the soil were not increased over the twelve-month period, but remained fairly constant.

The samples moistened with soil water appeared darker in the colorimeter than those containing only distilled water, but there were insufficient samples to warrant any conclusions being made.

Conclusions:

1. Soils having a relatively high organic matter content do not necessarily have the highest pigment content, nor are they always the darkest in color.

2. The second six inches of all samples studied were lower than the top six inches in relative pigment content.

3. The relative humus color of the soil extract is proportional to the relative pigment content.

4. In regions of approximately equal rainfall a sigmoid is obtained when organic matter content or relative humus content is plotted against mean annual temperature.

5. Traveling north or south along an isohyetal line, temperature has

a greater effect on organic matter and humus content of a soil in the region of highest precipitation.

6. Precipitation has a much more decided effect on organic matter, relative humus, and relative pigment content in the eastern portion of Nebraska than it has in the western part.

7. Generally speaking, it can be said that for every fall of $10^{\circ}C$. in mean annual temperature along the two isohyetal lines studied, the average:

(a) soil organic matter and humus content was about doubled.

(b) relative pigment content of the soil was increased from two to six times.

(c) relative humus color was increased from two to three times.

8. The effect of temperature along the two isohyets was greatest, in the one case, over eastern central Kansas and Oklahoma, and in the second, over western Nebraska, Kansas, and eastern Colorado.

9. With increasing precipitation along an isothermal line the average:

(a) soil organic matter, relative humus content, and relative pigment content, increased. The graphical nature of these relationships is a curve.

(b) relative humus color increased linearly.

10. With increasing temperature, along the isohyets, a linear decrease of relative humus color and relative pigment content was noted.

11. The relative humus color or relative pigment content of a soil was not increased by the addition of organic materials, and subsequent decomposition over a period of sixty weeks.

12. Of all soil groups studied the relative humus content was highest

in the podsol sample, followed in order by the gray-brown forest soils, the red and yellow soils, the chernozem samples and finally the laterite.

13. The relative pigment content and humus color, (excluding the surface soil of the podsol) were greatest for the chernozem soils, followed by the gray-brown forest soils, the laterite and red and yellow soils.

14. The laterite and red and yellow samples were fairly high in humus content, but low in relative pigment content.

Summary

1. A review of all the theories of pigment formation has been given.
2. Soil black pigments were isolated from gray-brown forest soils, grassland soils of the prairie, chernozem, and brown soil provinces, and from a muck soil.
3. Many of the physical and chemical properties of these pigments were determined.
4. The chemical reactions of acetylation, methylation, and base exchange were run on all three pigments.
5. Potentiometric and conductometric titration curves were plotted for the three pigments and their corresponding methylated and acetylated products.
6. Absorption capacities of the pigments and their derivatives were calculated from their respective potentiometric titration curves.
7. Quantitative differences between the pigments were pointed out.
8. A comparison of the chemical properties of the black pigments were made with those of plant and soil lignins.
9. Theories concerning the constitution of the black pigment in soil were considered.
10. A brief review of the literature concerning geographic distribution of the soil pigment and its relationship to climate was given.
11. The location and method of collection of all samples have been described.
12. Hygroscopic coefficient, hygroscopic moisture, per cent organic matter, and relative per cent humus, were determined on every soil sample.

13. The colorimetric method of determining humus was checked against the gravimetric method.

14. The effect of incubating organic materials in a Rosebud soil was studied with reference to its effect upon the black pigment content of that soil.

15. The relationships between mean annual temperature and per cent organic matter, relative humus and relative pigment contents and relative humus color have been shown.

16. A correlation between mean annual precipitation and organic matter, relative humus and relative pigment contents and relative humus color has been pointed out.

17. A comparison of the pigment and humus contents of soils from different soil groups or provinces has been made.

18. The different relationships between pigment content and humus color, and organic matter and relative pigment contents were clarified.

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Table 1.
Samples listed from north to south along eastern line of fairly constant N-S quotients.

| | | | | | | | | | | | | COUNTY AVERAGES | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Nearest Long-Time Weather Station | Mean Annual Precip-itation | Mean Annual Temper-ature (F) | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 347 | S. Dak. | Brown | Aberdeen | 25.39 | 43. | | 1.085 | 5.77 | 67. | 6.7 | 116. | 1.095 | 5.77 | 67. | 6.7 | 116. | 1.63 | 8.61 | 100 |
| 247 | S. Dak. | Day | Webster | 22.16 | 40.9 | | 1.79 | 5.94 | 115. | 14.4 | 120.5 | 1.79 | 5.94 | 115. | 14.4 | 120.5 | 1.24 | 4.12 | 80 |
| 387 | S. Dak. | Clark | Clark | 24.02 | 42.6 | | 1.77 | 5.88 | 128.5 | 15. | 136.5 | 1.77 | 5.88 | 128.5 | 15. | 136.5 | 1.18 | 3.92 | 86 |
| 5760 | S. Dak. | Brookings | | | | | | | | | | | | | | | | | |
| 4570 | S. Dak. | Brookings | Brookings | 20.23 | 43.5 | | 2.43 | 8. | 116. | 12.2 | 90. | | | | | | | | |
| 4568 | S. Dak. | Brookings | | | | | 2.12 | 7.20 | 113. | 3.0 | 101. | 2.28 | 7.60 | 114.5 | 12.2 | 95.5 | 1.86 | 6.22 | 94 |
| | S. Dak. | Beadler | Huron | 20.2 | 44. | 212 | | | | | | | | | | | | | |
| 297 | S. Dak. | Miner | Howard | 21.87 | 43.9 | | 2.55 | 6.97 | 187. | 15.5 | 137.5 | 2.55 | 6.97 | 187. | 15.5 | 137.5 | 1.64 | 4.49 | 121 |
| 337 | S. Dak. | Lake | | | | | 1.88 | 5.85 | 108.5 | 13.5 | 108.5 | 1.88 | 5.85 | 108.5 | 13.5 | 108.5 | 1.39 | 4.33 | 80 |
| 4576 | S. Dak. | McCook | Canistata | 20.76 | | | 1.88 | 5.22 | | | | | | | | | | | |
| 277 | S. Dak. | Hutchinson | | | | | 1.09 | 3.38 | 58.5 | 9.9 | 101.9 | 1.09 | 3.38 | 58.5 | 9.9 | 101. | 1.10 | 3.41 | 58 |
| 5819 | S. Dak. | Douglas | Armour | 22.94 | 47.3 | | 1.54 | 5.06 | 84. | 11.95 | 103. | 1.54 | 5.06 | 84. | 12. | 103. | 1.28 | 4.38 | 70 |
| | | Yankton | Yankton | 25.3 | 47. | 261 | | | | | | | | | | | | | |
| 2844 | Nebraska | Knox | | | | | | 5.40 | | | | | | | | | | | |
| 327 | (Eastern) | Knox | | | | | 1.09 | 3.71 | 49. | 12.2 | 84. | 1.09 | 4.55 | 49. | 12.2 | 84. | .89 | 3.72 | 40 |
| 2834 | (Eastern) | Cedar | Hartington | | 47.3 | | .81 | | 21. | 8.2 | 48. | | | | | | | | |
| 2839 | (Eastern) | Cedar | | | | | 1.13 | | 32. | 11.2 | 54. | .97 | | 26.5 | 9.7 | 51. | 1.00 | | 27 |
| 2828 | (Eastern) | Dixon | Wakefield | | 48.2 | | 1.13 | 4.17 | 32. | 9.4 | 52. | 1.13 | 4.17 | 32. | 9.4 | 52. | 1.16 | 4.43 | 34 |
| 2823 | (Eastern) | Dakota | | | | | | | | | | | | | | | | | |
| 3696 | (Eastern) | Pierce | | | | | 2.48 | | 121. | 12. | 72. | 2.48 | | 121. | 12. | 72. | 2.06 | | 101 |
| 4279 | (Eastern) | Madison | Madison | | 48.0 | | 1.09 | 5.07 | 20. | 12.9 | 35. | 1.09 | 5.07 | 20. | 12.9 | 35. | .84 | 3.93 | 16 |
| 4073 | (Eastern) | Cuming | West Point | | 49.8 | | 1.45 | 4.64 | 33. | 12.3 | 43. | 1.45 | 4.64 | 33. | 12.3 | 43. | 1.17 | 3.77 | 27 |
| 4083 | (Eastern) | Burt | Tekamah | | 49.6 | | 1.79 | 5.40 | 56. | 12.1 | 60. | | | | | | | | |
| 1373 | (Eastern) | Burt | | | | | 3.32 | 3.78 | 39. | 10.8 | 23. | 2.56 | 4.59 | 47.5 | 11.4 | 41.5 | 2.25 | 4.02 | 42 |
| 4281 | (Eastern) | Colfax | | | | | .91 | | 17. | 11.6 | 36. | .91 | | 17. | 11.6 | 36. | .78 | | 15 |
| 4065 | (Eastern) | Dodge | Fremont | | 49.5 | | | 6.16 | | | | | | | | | | | |
| 4788 | (Eastern) | Dodge | | | | | 1.46 | 5.0 | 58. | 11.5 | 75. | | | | | | | | |
| 2904 | (Eastern) | Dodge | | | | | 1.12 | 6.58 | 48. | 14.6 | 80. | | | | | | | | |
| 2907 | (Eastern) | Dodge | | | | | 2.99 | | 166.5 | 14.5 | 105. | | | | | | | | |
| 2970 | (Eastern) | Dodge | | | | | 2.28 | | 100.5 | 14.2 | 77. | 1.96 | 5.91 | 93.3 | 13.7 | 84.3 | 1.43 | 4.31 | 68 |
| 2878 | (Eastern) | Washington | Blair | | 49.9 | | 1.41 | | 41. | 10.5 | 56. | | | | | | | | |
| 2898 | (Eastern) | Washington | | 30. | | | 1.41 | 5.2 | 40. | 10.2 | 53. | 1.41 | 5.2 | 40.5 | 10.4 | 54.5 | 1.35 | 5.00 | 39 |
| 2918 | (Eastern) | Douglas | | | | | 1.49 | | 68. | 10.5 | 86. | | | | | | | | |
| 2920 | (Eastern) | Douglas | | | | | 1.68 | | 65. | 15.6 | 72. | | | | | | | | |
| 2922 | (Eastern) | Douglas | | | | | 1.58 | | 78. | 12.2 | 93. | 1.68 | | 70.3 | 12.8 | 83.6 | 1.31 | | 55 |
| | | Douglas | Omaha | 29. | 51. | 225 | | | | | | | | | | | | | |
| 1381 | (Eastern) | Saunders | | | | | 2.27 | 6.19 | 98. | 12.2 | 79. | | | | | | | | |
| 1393 | (Eastern) | Saunders | | | | | 1.62 | 4.58 | 67. | 11.4 | 77. | | | | | | | | |
| 2557 | (Eastern) | Saunders | | | | | | | | | | | | | | | | | |
| 2750 | (Eastern) | Saunders | | | | | 2.28 | 5.91 | 86. | 11.1 | 72. | | | | | | | | |

| | | | | | | | | | | | | COUNTY AVERAGES | | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Nearest Long-Time Weather Station | Mean Annual Precip-itation | Mean Annual Temper-ature (F) | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 4197 | (Eastern) | Saunders | | | | | 1.15 | 5.22 | 26. | 11.1 | 42. | | | | | | | | |
| 2771 | (Eastern) | Saunders | | | | | 2.30 | 6.0 | 95. | 11.1 | 78. | 1.92 | 5.47 | 74.4 | 11.4 | 69.8 | 1.68 | 4.79 | 65 |
| 4669 | (Eastern) | Butler | | | | | 1.69 | 5.68 | 94. | | 105. | | | | | | | | |
| 4675 | (Eastern) | Butler | | | | | 2.04 | 5.84 | 123. | | 113. | | | | | | | | |
| 2960 | (Eastern) | Butler | | | | | 2.69 | 6.40 | 90. | 11.8 | 80. | | | | | | | | |
| 2729 | (Eastern) | Butler | | | | | 2.01 | 5.3 | 79. | 10.7 | 74. | 2.11 | 5.80 | 96.5 | 11.3 | 93. | 1.86 | 5.13 | 85 |
| 2456 | Nebraska | Polk | | | | | .68 | 3.2 | 14. | 9.6 | 38. | .68 | 3.20 | 14. | 9.6 | 38. | .70 | 3.33 | 15 |
| 4657 | Nebraska | Seward | | | | | .20 | 6.18 | 11. | 10.5 | 105. | | | | | | | | |
| 2617 | Nebraska | Seward | | | | | 1.41 | 5.14 | 41.5 | 11.6 | 54. | | | | | | | | |
| 2610 | Nebraska | Seward | | | | | 2.41 | | | 10. | 88. | 1.34 | 5.66 | 21.2 | 10.7 | 82.3 | 1.25 | 5.28 | 20 |
| 4696 | Nebraska | Lancaster | | | | | 1.53 | | 75. | 9.0 | 93. | | | | | | | | |
| 4762 | Nebraska | Lancaster | Lincoln | | | 205 | | | | | | | | | | | | | |
| 4778 | Nebraska | Lancaster | | | | | .79 | | 10. | | 23.5 | | | | | | | | |
| 4698 | Nebraska | Lancaster | | | | | 1.79 | | 92. | 11.5 | 97. | | | | | | | | |
| 4776 | Nebraska | Lancaster | | | | | 1.58 | | 69. | 11.8 | 83. | 1.42 | | | | | | | |
| 2694 | Nebraska | Cass | | | | | 2.17 | 5.70 | 116. | 10.8 | 101. | | | 61.5 | 1.08 | 74.1 | 1.31 | | 57 |
| 2701 | Nebraska | Cass | Weeping Water | | 51.1 | | 2.01 | 5.40 | 81. | 9.8 | 76. | | | | | | | | |
| 2743 | Nebraska | Cass | | | | | 2.41 | 6.18 | 109 | 11. | 86. | | | | | | | | |
| 2736 | Nebraska | Cass | | | | | 2.45 | 6.20 | 120. | 11.6 | 92. | | | | | | | | |
| 3700 | Nebraska | Cass | | | | | 1.56 | 6.22 | 65. | 11.1 | 79. | | | | | | | | |
| 3710 | Nebraska | Cass | | | | | 1.63 | 4.72 | 60. | 10.6 | 69. | | | | | | | | |
| 2708 | Nebraska | Cass | | | | | 1.87 | 5.33 | 74. | 10.4 | 76. | 2.01 | 5.67 | 89.3 | 10.8 | 82.7 | 1.86 | 5.25 | 83 |
| 4415 | Nebraska | Clay | | | | | 1.49 | 4.3 | 70. | 9.8 | 88. | | | | | | | | |
| 4401 | Nebraska | Clay | | | | | 1.63 | 4.4 | 90. | 8.5 | 104. | | | | | | | | |
| 4407 | Nebraska | Clay | Clay Center | | 50.8 | | | | | | | | | | | | | | |
| 4413 | Nebraska | Clay | | | | | 1.56 | 4.2 | 86. | 8.5 | 104. | | | | | | | | |
| 5766 | Nebraska | Clay | | | | | 1.51 | | 84. | 9.4 | 104. | 1.55 | 4.3 | 82.5 | 9.1 | 100. | 1.70 | 4.20 | 81 |
| 4784 | Nebraska | Saline | | | | | 1.24 | 4.66 | 90. | 11.1 | 136. | 1.24 | 4.66 | 90. | 11.1 | 136. | 1.11 | | |
| 4475 | Nebraska | Otoe | | | | | 1.22 | | 48.5 | 9.2 | 75. | | | | | | | | |
| 4465 | Nebraska | Otoe | | | | | .83 | | 12. | | 28. | | | | | | | | |
| 4455 | Nebraska | Otoe | | | | | 1.53 | 5.00 | 79.5 | | 86. | | | | | | | | |
| 2856 | Nebraska | Otoe | | | | | 2.03 | 5.4 | 83. | 11.4 | 77. | 1.40 | 5.2 | 55.8 | 10.3 | 66.5 | 1.35 | 5.04 | 54 |
| 1-11 | Nebraska | Nuckolls | Superior | | 51.8 | | 1.24 | 4.3 | | 9.4 | 85. | 1.24 | 4.3 | | 9.4 | 85. | 1.31 | 4.57 | |
| 1-12 | Nebraska | Thayer | | | | | .91 | 4.8 | | 9.7 | 64. | .91 | 4.8 | | 9.7 | 64. | .93 | 4.94 | |
| 4649 | Nebraska | Jefferson | | | | | 2.14 | 6.24 | 127. | 11. | 112. | | | | | | | | |
| 4639 | Nebraska | Jefferson | | | | | 1.78 | 5.00 | 104. | 8.6 | 110. | 1.96 | 5.62 | 115.5 | 9.8 | 111. | 2.00 | 5.73 | 118 |
| 3720 | Nebraska | Gage | | | | | 1.66 | 5.78 | 96. | 10.2 | 110. | 1.66 | 4.8 | 96. | 10.2 | 110. | 1.62 | 4.70 | 94 |
| 2496 | Nebraska | Gage | | | | | | 3.8 | | | | | | | | | | | |
| 2574 | Nebraska | Johnson | | | | | 1.86 | 5.56 | 90. | 11.2 | 91. | | | | | | | | |
| 4439 | Nebraska | Johnson | Tecumseh | | 51.7 | | | 4.70 | | | | | | | | | | | |

| | | | | | | | | | | | | COUNTY AVERAGES | | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Nearest Long-Time Weather Station | Mean Annual Precip-itation | Mean Annual Temper-ature (F) | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 4896 | Nebraska | Box Butte | | | | | 1.06 | 4.5 | 46. | 9.0 | 81 | | | | | | | | |
| 4976 | Nebraska | Box Butte | | | | | .94 | 3.4 | 46. | 9.6 | 91 | | | | | | | | |
| 4968 | Nebraska | Box Butte | | | | | 1.08 | 3.3 | 52. | 9.2 | 91 | | | | | | | | |
| 4972 | Nebraska | Box Butte | | | | | 1.0 | 3.5 | 42. | 8.8 | 80 | | | | | | | | |
| 4940 | Nebraska | Box Butte | | | | | .91 | | 36. | 7.4 | 80 | 0.85 | 2.95 | 35.5 | 7.28 | 77.5 | 1.18 | 3.77 | 49 |
| 4878 | Nebraska | Scottsbluff | | | | | .35 | | 8.5 | 6.2 | 45 | | | | | | | | |
| 4884 | Nebraska | Scottsbluff | Mitchell | 16″ | 46.6 | | .34 | | 7. | 3.75 | 39.5 | | | | | | | | |
| 4888 | Nebraska | Scottsbluff | | \| | | | .68 | 2.6 | 17.5 | 8.4 | 49 | 0.45 | 2.6 | 11. | 6.11 | 44.5 | .73 | | 18 |
| 3866 | Nebraska | Morrill | | \| | | | 3.88 | | 76. | 5.9 | 37 | | | | | | | | |
| 3879 | Nebraska | Morrill | Bridgeport | \| | 47.8 | | .60 | | 15. | 6.5 | 45 | 2.24 | | 45.5 | 6.20 | 41. | 3.61 | | 73 |
| 4822 | Nebraska | Banner | | \| | | | .80 | 2.8 | 37. | 7.8 | 88 | | | | | | | | |
| 4874 | Nebraska | Banner | | \| | | | | | | | | | | | | | | | |
| 4826 | Nebraska | Banner | | \| | | | .84 | 3.6 | 29. | 7.4 | 65 | | | | | | | | |
| 4830 | Nebraska | Banner | | \| | | | | | | | | .82 | 3.2 | 33. | 7.6 | 76.5 | 1.07 | 4.21 | 43 |
| 2944 | Nebraska | Kimball | | \| | | | | 2.5 | | | | | | | | | | | |
| 3987 | Nebraska | Kimball | | \| | | | .99 | 2.8 | 36. | 7.7 | 68 | | | | | | | | |
| 4834 | Nebraska | Kimball | | \| | | | .37 | 2.1 | 19. | 6.0 | 75 | | | | | | | | |
| 4838 | Nebraska | Kimball | Kimball | \| | 48.3 | | .77 | 3.1 | 30. | 6.6 | 74 | | | | | | | | |
| 4814 | Nebraska | Kimball | | \| | | | .71 | | 28. | 5.6 | 73 | | | | | | | | |
| 4818 | Nebraska | Kimball | | \| | | | .57 | 2.1 | | 6.5 | 68 | | | | | | | | |
| 4842 | Nebraska | Kimball | | to | | | | | | | | | | | | | | | | |
| 4846 | Nebraska | Kimball | | \| | | | .66 | 3.1 | 24. | 7.2 | 71 | | | | | | | | |
| 4850 | Nebraska | Kimball | | \| | | | 1.21 | | 62. | 11.2 | 97 | | | | | | | | |
| 4854 | Nebraska | Kimball | | \| | | | .60 | | 21. | 5.55 | 66 | | | | | | | | |
| 4858 | Nebraska | Kimball | | \| | | | .62 | 2.4 | 22. | | 68 | | | | | | | | |
| 4862 | Nebraska | Kimball | | \| | | | .75 | | 20. | 8.15 | 50 | | | | | | | | |
| 4866 | Nebraska | Kimball | | \| | | | .88 | | 38.5 | 9.35 | 82 | | | | | | | | |
| 4870 | Nebraska | Kimball | | \| | | | .61 | | 19.5 | 6.0 | 61 | .72 | 2.58 | 29.09 | 7.2 | 71. | 1.00 | 3.58 | 40 |
| 4800 | Nebraska | Cheyenne | | \| | | | .62 | | 24. | 6.45 | 72 | | | | | | | | |
| 4808 | Nebraska | Cheyenne | Lodgepole | \| | 48.2 | | .94 | 2.21 | 29. | | 61 | | | | | | | | |
| 3993 | Nebraska | Cheyenne | | \| | | | | 3.13 | | | | | | | | | | | |
| 4001 | Nebraska | Cheyenne | | \| | | | .99 | 3.0 | 44. | 9.1 | 84 | | | | | | | | |
| 4007 | Nebraska | Cheyenne | | \| | | | | 3.5 | | | | | | | | | | | |
| 4804 | Nebraska | Cheyenne | | \| | | | .74 | 2.9 | 35. | 8.5 | 90 | .82 | 2.94 | 33. | 8.0 | 76.75 | 1.02 | 3.67 | 41 |
| 4011 | Nebraska | Duel | | \| | 48.5 | | .91 | 2.7 | 31. | 7.1 | 66 | | | | | | | | |
| 4013 | Nebraska | Duel | | \| | | | 1.21 | 3.3 | 56. | 8.5 | 86 | 1.06 | 3.0 | 43.5 | 7.8 | 76. | 1.36 | 3.84 | 56 |
| 4019 | Nebraska | Perkins | Grant | \| | 48.7 | | 1.29 | | 67. | 8.5 | 97 | | | | | | | | |
| 4023 | Nebraska | Perkins | Madrid | 20″ | 49.5 | | 1.52 | | 73. | 9.9 | 90 | 1.40 | | 70. | 9.2 | 93.5 | 1.52 | | 76 |
| 1-5 | Colorado | Weld | Grover | 13.84 | 45.7 | | .85 | 2.5 | 32. | 9.0 | 70 | .85 | 2.5 | 32. | 9.0 | 70. | .90 | 2.77 | 36 |
| 1-4 | Colorado | Logan | | | | | .40 | 1.9 | 14. | 4.2 | 66 | .40 | 1.9 | 14. | 4.2 | 66. | .95 | 4.52 | 33 |

| | | | | | | | | | | | | COUNTY AVERAGES | | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Nearest Long-Time Weather Station | Mean Annual Precip-itation | Mean Annual Temper-ature (F) | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 39 | Colorado | Sedgwick | Sedgwick | 17.54 | 48.4 | | .32 | 1.08 | 12. | 2.9 | 72 | .32 | 1.08 | 12. | 2.9 | 72. | 1.13 | 3.72 | 41 |
| 38 | Colorado | Phillips | Haxtun | 16.97 | | | .47 | 1.56 | 14. | 3.5 | 58 | .47 | 1.56 | 14. | 3.5 | 58. | 1.34 | 4.45 | 40 |
| 237 | Colorado | Yuma | Wray | 17.44 | 50.7 | | .43 | 1.48 | 15. | 4.8 | 68 | | | | | | | | |
| 207 | Colorado | Yuma | | | | | .33 | 1.78 | 8. | 3.5 | 48.5 | | | | | | | | |
| 37 | Colorado | Yuma | | | | | .53 | 1.67 | 22. | 4.7 | 78 | .43 | 1.64 | 15. | 4.33 | 64.8 | .99 | 3.78 | 35 |
| 36 | Colorado | Kit Carson | Burlington | 17.59 | 50.4 | | .36 | 1.21 | 13. | 6.6 | 71 | .36 | 1.21 | 13. | 6.6 | 71. | .54 | 2.20 | 20 |
| 4031 | Nebraska | Chase | Imperial | Ranges | 49.7 | | 1.08 | 3.24 | 49. | 9.0 | 85 | | | | | | | | |
| 4037 | Nebraska | Chase | | from | | | .81 | 2.57 | 29. | 8.0 | 62 | | | | | | | | |
| 4025 | Nebraska | Chase | | 18" | | | .81 | 2.22 | 35. | 6.6 | 82 | .90 | 2.34 | 37.6 | 7.86 | 76. | 1.14 | 3.0 | 48 |
| 3630 | Nebraska | Hayes | | \| | | 1.07 | | 44. | 8.3 | 78 | | | | | | | | |
| 137 | Nebraska | Hayes | | to | | 143 | .96 | 3.13 | 46. | 8.5 | 91 | 1.01 | 3.13 | 45. | 8.4 | 84.5 | 1.20 | 3.72 | 54 |
| 4039 | Nebraska | Hitchcock | Culbertson | \| | 51.0 | | 1.08 | | 52. | 9.5 | 91 | 1.08 | | 52. | 9.5 | 91. | 1.13 | | 55 |
| 367 | Kansas | Cheyenne | Benkelman, Nebraska | 20" | 50.8 | | .49 | 2.15 | 20. | 9.8 | 78 | | | | | | | | |
| 127 | Kansas | Cheyenne | | | | | .60 | 2.44 | 27.5 | 9.3 | 86.5 | | | | | | | | |
| 67 | Kansas | Cheyenne | | | | | .74 | 2.61 | 40.5 | 9.8 | 103 | | | | | | | | |
| 27 | Kansas | Cheyenne | | | | | 1.05 | 3.16 | 48. | 9.1 | 87 | .72 | 2.59 | 29.5 | 9.5 | 88.6 | .75 | 2.72 | 31 |
| 34 | Kansas | Thomas | Colby | 18.44 | 51.8 | | .66 | 1.68 | 33. | 10.4 | 96 | .66 | 1.68 | 33. | 10.4 | 96. | .63 | 1.61 | 32 |
| 35 | Kansas | Sherman | Goodland | 18.71 | | | .74 | 2.49 | 37. | 9.8 | 95 | .74 | 2.49 | 37. | 9.8 | 95. | .75 | 2.54 | 38 |
| 33 | Kansas | Scott | Scott City | 19.93 | | | .99 | 3.50 | 49. | 15.3 | 93 | .99 | 3.50 | 49. | 15.3 | 93. | .64 | 2.28 | 32 |
| 4371 | Kansas | Trego | Hays | 22.91 | 53.5 | | .82 | 3.00 | 32. | 9.90 | 73 | | | | | | | | |
| 4373 | Kansas | Trego | | | | | .93 | 3.37 | 40. | 9.45 | 83 | .87 | 3.51 | 36. | 9.68 | 78. | .89 | 3.62 | 37 |
| 4361 | Kansas | Ellis | Wakeeney | 21.15 | 53.7 | | .77 | 3.47 | 26. | 11.75 | 63 | | | | | | | | |
| 4365 | Kansas | Ellis | | | | | 1.05 | 4.06 | 44. | 11.60 | 79 | .91 | 3.76 | 35. | 11.68 | 71. | .77 | 3.21 | 30 |
| 32 | Kansas | Finney | Garden City | 19.48 | 54.4 | | .59 | 2.10 | 21. | 7.6 | 68 | .59 | 2.10 | 21. | 7.6 | 68. | .77 | 2.76 | 28 |
| 5758 | Kansas | Ford | Dodge City | 20.4 | 54.0 | 123 | .95 | 3.15 | 51. | | 103 | .95 | 3.15 | 51. | | 103. | .90 | 3.00 | 47 |
| 31 | Kansas | Seward | Liberal | 19.08 | | | | 2.59 | | 7.2 | | | | | 7.2 | | | 3.59 | |
| 1-7 | Colorado | Prowers | Holly | 15.09 | 53.6 | | .51 | 1.9 | 21. | 7.7 | 75 | .51 | 1.9 | 21. | 7.7 | 75. | .66 | 2.46 | 27 |
| 1-8 | Colorado | Oter | | | | | .38 | 2.2 | 55. | 11.2 | 28 | .38 | 2.2 | 55. | 11.2 | 28. | .33 | 1.96 | 49 |
| 1-9 | Colorado | Las Animas | | | | | .47 | 1.8 | 15. | 9.6 | 61 | .47 | 1.8 | 15. | 9.6 | 61. | .48 | 1.87 | 16 |
| 1-6 | Texas | | | | | | .47 | 1.7 | 17. | 4.3 | 67 | .47 | 1.7 | 17. | 4.3 | 67. | 1.09 | 3.95 | 40 |
| 30 | Texas | Carson | | | | | 1.65 | 1.8 | 13. | 6.6 | 14 | | | | | | | | |
| 29 | Texas | Carson | | | 58.2 | | .81 | 2.43 | 38. | 10.5 | 89 | | | | | | | | |
| 28 | Texas | Carson | | | 59.2 | | .75 | 2.44 | 37. | 8.5 | 93 | 1.07 | 2.22 | 29. | 8.5 | 65. | 1.25 | 2.61 | 34 |
| 27 | Texas | Armstrong | | | 59.5 | | 1.88 | 2.8 | 26. | 8.9 | 26 | 1.88 | 2.8 | 26. | 8.9 | 26. | 2.11 | 3.14 | 29 |
| 26 | Texas | Donley | | | 62.3 | | 1.84 | .9 | 7. | 4.3 | 7 | 1.84 | .9 | 7. | 4.3 | 7. | 4.27 | 2.09 | 16 |
| 25 | Texas | Wichita | | | 65.1 | | 1.68 | 1.25 | 24. | 6.2 | 27 | 1.68 | 1.25 | 24. | 6.2 | 27. | 2.70 | 2.01 | 39 |

Table 3
Samples listed from east to west along an isotherm

| | | | | | | | | | | | COUNTY AVERAGES | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Mean Annual Precip-itation | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 4083 | Nebraska | Burt | | | 1.79 | 5.40 | 56 | 12.1 | 60 | | | | | | | | |
| 1373 | Nebraska | Burt | 30″ | | 3.37 | 3.78 | 39 | 10.8 | 23 | 2.58 | 4.59 | 42.5 | 11.5 | 41.5 | 2.24 | 3.99 | 37 |
| 2878 | Nebraska | Washington | 28-30″ | | 1.41 | | 41 | 10.5 | 56 | | | | | | | | |
| 2898 | Nebraska | Washington | | | 1.41 | 5.2 | 40 | 10.2 | 53 | 1.41 | 5.2 | 40.5 | 10.3 | 54.5 | 1.36 | 5.04 | 39 |
| 2918 | Nebraska | Douglas | 30-32″ | | 1.49 | | 68 | 10.5 | 86 | | | | | | | | |
| 2920 | Nebraska | Douglas | | 225 | 1.68 | | 65 | 15.6 | 72 | | | | | | | | |
| 2922 | Nebraska | Douglas | | | 1.58 | | 78 | 12.2 | 93 | 1.58 | | 70. | 12.8 | 83.6 | 1.23 | | 55 |
| 4065 | Nebraska | Dodge | 28-30″ | | 1.80 | 6.16 | 74 | 12.6 | 77 | | | | | | | | |
| 4788 | Nebraska | Dodge | | | 1.47 | 5.0 | 58 | 11.5 | 75 | | | | | | | | |
| 2904 | Nebraska | Dodge | | | | 6.58 | | | | | | | | | | | |
| 2907 | Nebraska | Dodge | | | 1.12 | 7.05 | 48 | 14.6 | 80 | | | | | | | | |
| 2970 | Nebraska | Dodge | | | 2.28 | 6.72 | 101 | 14.2 | 77 | 1.67 | 6.30 | 70. | 13.2 | 77. | 1.26 | 4.77 | 53 |
| 1381 | Nebraska | Saunders | 28-30″ | 205 | 2.32 | 6.19 | 98 | 12.2 | 79 | | | | | | | | |
| 1393 | Nebraska | Saunders | | | 1.62 | 4.58 | 67 | 11.4 | 77 | | | | | | | | |
| 2757 | Nebraska | Saunders | | | 1.96 | 5.29 | 75 | 11.0 | 73 | | | | | | | | |
| 2750 | Nebraska | Saunders | | | 2.28 | 5.91 | 86 | 11.1 | 72 | | | | | | | | |
| 4197 | Nebraska | Saunders | | | 1.15 | 5.22 | 26 | 11.1 | 42 | | | | | | | | |
| 2771 | Nebraska | Saunders | | | 2.30 | 6.0 | 95 | 11.1 | 78 | 1.94 | 5.53 | 75 | 11.3 | 59. | 1.71 | 4.89 | 66 |
| 4669 | Nebraska | Butler | 28-30″ | | 1.69 | 5.68 | 94 | | 105 | | | | | | | | |
| 4675 | Nebraska | Butler | | | 2.04 | 5.84 | 123 | | 113 | | | | | | | | |
| 2960 | Nebraska | Butler | | | 2.69 | 6.40 | 90 | 11.8 | 80 | | | | 11.3 | 93. | 1.86 | 5.13 | 86 |
| 2729 | Nebraska | Butler | | | 2.01 | 5.3 | 79 | 10.7 | 74 | 2.11 | 5.80 | 97 | | | | | |
| 2456 | Nebraska | Polk | 26-28″ | | .68 | 3.2 | 14 | 9.6 | 38 | .68 | 3.2 | 14 | 9.6 | 38. | .70 | 3.33 | 15 |
| 1316 | Nebraska | Hall | 24-26″ | | 1.20 | 3.96 | 50 | 9.7 | 67 | | | | | | | | |
| 2646 | Nebraska | Hall | | | 1.14 | 2.41 | 68 | 7.7 | 117 | | | | | | | | |
| 2669 | Nebraska | Hall | | | 1.93 | 4.36 | 90 | 8.8 | 88 | | | | | | | | |
| 2623 | Nebraska | Hall | | | 1.06 | 2.95 | 48 | 8.2 | 84 | | | | | | | | |
| 2677 | Nebraska | Hall | | | 1.13 | 3.78 | 42 | 5.7 | 70 | | | | | | | | |
| 2673 | Nebraska | Hall | | | .76 | 2.75 | 28 | 4.1 | 69 | | | | | | | | |
| 1302 | Nebraska | Hall | | | 1.64 | 4.05 | 81 | 3.2 | 93 | | | | | | | | |
| 2632 | Nebraska | Hall | | | .48 | 1.31 | 13 | 2.7 | 51 | 1.17 | 3.19 | 53 | 6.3 | 80. | 1.85 | 5.06 | 84 |
| 3808 | Nebraska | Howard | 24-26″ | | 1.19 | 3.74 | 48 | 9.2 | 75 | | | | | | | | |
| 1284 | Nebraska | Howard | | | .64 | 1.59 | 23 | 4.0 | 67 | | | | | | | | |
| 1286 | Nebraska | Howard | | | .46 | 1.41 | 9 | 2.6 | 40 | .76 | 2.24 | 27 | 5.3 | 60. | 1.43 | 4.22 | 51 |
| 3812 | Nebraska | Sherman | 22-24″ | | .92 | 3.64 | 23 | 9.4 | 46 | .92 | 3.64 | 23 | 9.4 | 46. | .97 | 3.87 | 24 |
| 4395 | Nebraska | Kearney | 26-28″ | | 1.08 | 3.5 | 44 | 8.6 | 76 | | | | | | | | |
| 4349 | Nebraska | Kearney | | | 1.10 | 3.5 | 42 | 7.9 | 71 | | | | | | | | |
| 4351 | Nebraska | Kearney | | | 1.41 | 4.2 | 68 | 8.8 | 91 | | | | | | | | |
| 4353 | Nebraska | Kearney | | | 1.20 | 3.8 | 53 | 8.6 | 83 | | | | | | | | |
| 4359 | Nebraska | Kearney | | | 1.17 | 4.0 | 52 | 8.3 | 84 | 1.19 | 3.8 | 52 | 8.5 | 81. | 1.40 | 4.47 | 61 |
| 3839 | Nebraska | Custer | 22-24″ | | 1.38 | | | 7.6 | 71 | | | | | | | | |

| | | | | | | | | | | | COUNTY AVERAGES | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Mean Annual Precip-itation | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 3847 | Nebraska | Custer | | | 1.38 | 4.07 | 51 | 7.2 | 70 | | | | | | | | |
| 2519 | Nebraska | Custer | | | | | | | | 1.38 | 4.07 | 51 | 7.4 | 71. | 1.86 | 5.50 | 69 |
| 2884 | Nebraska | Dawson | 20-22″ | 143 | .71 | 2.16 | 18 | 10.8 | 48 | .71 | 2.16 | 18 | 10.8 | 48. | .65 | 2.00 | 17 |
| 3630 | Nebraska | Hayes | 22-24″ | | 1.07 | | 44 | 8.3 | 78 | | | | | | | | |
| 137 | Nebraska | Hayes | | | .96 | 3.13 | 46 | 8.5 | 91 | 1.02 | 3.13 | 45 | 8.4 | 85. | 1.21 | 3.72 | 54 |
| 4031 | Nebraska | Chase | 18-20″ | | 1.08 | 3.24 | 49 | 9.0 | 85 | | | | | | | | |
| 4037 | Nebraska | Chase | | | .87 | 2.57 | 29 | 8.0 | 62 | | | | | | | | |
| 4025 | Nebraska | Chase | | | .81 | 2.22 | 35 | 6.6 | 82 | .92 | 2.67 | 38 | 7.9 | 76. | 1.16 | 3.37 | 48 |
| 4019 | Nebraska | Perkins | 18-20″ | | 1.29 | 3.47 | 67 | 8.5 | 97 | | | | | | | | |
| 4023 | Nebraska | Perkins | | | 1.52 | 3.86 | 73 | 9.9 | 90 | 1.41 | 3.66 | 70 | 9.2 | 94. | 1.53 | 3.97 | 76 |
| 4011 | Nebraska | Duel | 18″ | | .90 | 2.7 | 31 | 7.1 | 66 | | | | | | | | |
| 4013 | Nebraska | Duel | | | 1.21 | 3.3 | 56 | 8.5 | 86 | 1.06 | 3.0 | 44 | 7.8 | 76. | 1.35 | 3.84 | 56 |
| 4800 | Nebraska | Cheyenne | 16-18″ | | .62 | 2.21 | 24 | 6.45 | 72 | | | | | | | | |
| 4808 | Nebraska | Cheyenne | | | .92 | 3.13 | 29 | | 61 | | | | | | | | |
| 3993 | Nebraska | Cheyenne | | | 1.14 | | 44 | 8.8 | 72 | | | | | | | | |
| 4001 | Nebraska | Cheyenne | | | .99 | 3.0 | 44 | 9.1 | 84 | | | | | | | | |
| 4007 | Nebraska | Cheyenne | | | 1.15 | 3.5 | 38 | 9.0 | 63 | | | | | | | | |
| 4804 | Nebraska | Cheyenne | | | .74 | 2.9 | 35 | 8.5 | 90 | | | | | | | | |
| 2944 | Nebraska | Cheyenne | | | .70 | 2.5 | 23 | 5.2 | 63 | .89 | 2.87 | 34 | 7.9 | 72. | 1.12 | 3.63 | 43 |
| 3987 | Nebraska | Kimball | 17″ | | .99 | 2.8 | 36 | 7.7 | 68 | | | | | | | | |
| 4834 | Nebraska | Kimball | | | .47 | 2.1 | 19 | 6.0 | 75 | | | | | | | | |
| 4838 | Nebraska | Kimball | | | .77 | 3.1 | 30 | 6.6 | 74 | | | | | | | | |
| 4814 | Nebraska | Kimball | | | .71 | | 28 | 5.6 | 73 | | | | | | | | |
| 4818 | Nebraska | Kimball | | | .57 | 2.1 | 21 | 6.5 | 68 | | | | | | | | |
| 4846 | Nebraska | Kimball | | | .66 | 3.1 | 24 | 7.2 | 71 | | | | | | | | |
| 4850 | Nebraska | Kimball | | | 1.21 | | 62 | 11.2 | 97 | | | | | | | | |
| 4854 | Nebraska | Kimball | | | .60 | | 21 | 5.55 | 66 | | | | | | | | |
| 4858 | Nebraska | Kimball | | | .62 | 2.4 | 22 | | 68 | | | | | | | | |
| 4862 | Nebraska | Kimball | | | .75 | | 20 | 8.15 | 50 | | | | | | | | |
| 4866 | Nebraska | Kimball | | | .88 | | 38 | 9.35 | 82 | | | | | | | | |
| 4870 | Nebraska | Kimball | | | .61 | | 20 | 6.0 | 61 | .74 | 2.60 | 29 | 7.3 | 71. | 1.01 | 3.56 | 40 |
| 4822 | Nebraska | Banner | 16″ | | .80 | 2.8 | 37 | 7.8 | 88 | | | | | | | | |
| 4826 | Nebraska | Banner | | | .85 | 3.6 | 29 | 7.4 | 65 | .83 | 3.2 | 33 | 7.6 | 77. | 1.09 | 4.21 | 43 |
| 1-4 | Colorado | Logan | 17.6″ | | .40 | 1.9 | 14 | 4.2 | 66 | .57 | 2.16 | 26 | 6.5 | 80. | .87 | 3.32 | 40 |
| 39 | Colorado | Sedgwick | 17.4″ | | .32 | 1.08 | 12 | 2.9 | 72 | .32 | 1.08 | 12 | 2.9 | 72. | 1.10 | 3.72 | 41 |
| 38 | Colorado | Phillips | 17.6″ | | .47 | 1.56 | 14 | 3.5 | 58 | .47 | 1.56 | 14 | 3.5 | 58. | 1.34 | 4.45 | 40 |
| 37 | Colorado | Yuma | 17.4″ | | .53 | 1.67 | 22 | 4.7 | 78 | | | | | | | | |
| 237 | Colorado | Yuma | | | .43 | 1.48 | 15 | 4.8 | 68 | | | | | | | | |
| 207 | Colorado | Yuma | | | .33 | 1.78 | 8 | 3.5 | 48.5 | .43 | 1.64 | 15 | 4.3 | 64. | 1.00 | 3.81 | 35 |
| 1-5 | Colorado | Weld | | | .85 | 2.5 | 32 | 9.0 | 70 | .85 | 2.5 | 32 | 9.0 | 70. | .94 | 2.77 | 36 |
| Denver, | Colorado | | 13.85 | 76 | | | | | | | | | | | | | |
| 3963 | Wyoming | Laramie | | | .58 | 1.8 | 16 | 5.5 | 50 | | | | | | | | |
| 3965 | Wyoming | Laramie | | | .63 | 2.3 | 13 | 5.2 | 38 | | | | | | | | |
| 3971 | Wyoming | Laramie | | | .70 | 2.7 | 21 | 7.3 | 57 | .64 | 2.2 | 17 | 6.0 | 48. | 1.06 | 3.66 | 30 |

| | | | | | | | | | | COUNTY AVERAGES | | | | | County Averages Expressed on an equivalent textural basis: HC=10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample number | State | County | Mean Annual Precip-itation | N.S.Q. | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content | Hygro-scopic Coeffi-cient | Relative Humus Color | Per cent Humus Extracted | Per cent Organic Matter | Relative Pigment Content |
| 3626 | Kansas | Marion | | | 2.36 | | 36 | 12.9 | 29 | | | | | | | | |
| 5 | Kansas | Marion | | 183 | 1.54 | 4.71 | 88 | 10.2 | 107 | | | | | | | | |
| 6 | Kansas | Marion | 30.35 | | 1.14 | 5.6 | 56 | 16.1 | 93 | | | | | | | | |
| 7 | Kansas | Marion | | | 1.24 | 4.34 | 59 | 9.9 | 89 | 1.57 | 4.9 | 60 | 12.3 | 80 | 1.27 | 3.98 | 49 |
| 8 | Kansas | Butler | | | 1.28 | 4.95 | 54 | 11.6 | 79 | 1.28 | 4.95 | 54 | 11.6 | 79 | 1.10 | 4.26 | 47 |
| 4317 | Kansas | McPherson | | | 1.25 | 3.70 | 66 | 8.0 | 100 | 1.25 | 3.7 | 66 | 8.0 | 100 | 1.56 | 4.62 | 83 |
| 3606 | Kansas | Sedgwick | 30.14 | | 1.56 | | 65 | 8.4 | 78 | 1.56 | | 65 | 8.4 | 78 | 1.85 | | 77 |
| 4361 | Kansas | Ellis | | | .77 | 3.47 | 26 | 11.75 | 63 | | | | | | | | |
| 4365 | Kansas | Ellis | | | 1.05 | 4.06 | 44 | 11.60 | 79 | .91 | 3.77 | 35 | 11.7 | 71 | .77 | 3.22 | 30 |
| 4371 | Kansas | Trego | | | .82 | 3.60 | 32 | 9.90 | 73 | | | | | | | | |
| 4373 | Kansas | Trego | | | .93 | 3.37 | 40 | 9.45 | 83 | .88 | 3.49 | 36 | 9.7 | 78 | .90 | 3.59 | 37 |
| 33 | Kansas | Scott | 19.93 | | .99 | 3.50 | 49 | 15.3 | 93 | .99 | 3.50 | 49 | 15.3 | 93 | .64 | 2.28 | 32 |
| 5758 | Kansas | Ford | 20.4 | 123 | .95 | 3.15 | 51 | | 103 | .95 | 3.13 | 51 | | 103 | | | |
| 32 | Kansas | Finney | 19.5 | | .59 | 2.10 | 21 | 7.6 | 68 | .59 | 2.10 | 21 | 7.6 | 68 | .77 | 2.76 | 28 |
| 31 | Kansas | Seward | | | 2.59 | | | 7.2 | | 2.59 | | | 7.2 | | 3.59 | | |
| 1-7 | Colorado | Prowers | 15. | | .51 | 1.90 | 21 | 7.7 | 75 | .51 | 1.90 | 21 | 7.7 | 75 | .66 | 2.46 | 28 |
| 1-8 | Colorado | Otero | | | .38 | 2.2 | 5.5 | 11.2 | 28 | .38 | 2.20 | 5.5 | 11.2 | 28 | .33 | 1.96 | 5 |
| 1-9 | Colorado | Las Animas | | | .47 | 1.8 | 15 | 9.6 | 61 | .47 | 1.80 | 15 | 9.6 | 61 | .48 | 1.87 | 16 |
| | | (Bent—Las Animas) | 12.5 | 81 | | | | | | | | | | | | | |

# APPENDIX

TABLE 1A

ALONG EASTERN ISOHYET

| State | Mean Annual Temperature | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|
| | | 116 | 1.63 | 8.61 | 100 |
| | | 121 | 1.24 | 4.12 | 80 |
| South | | 137 | 1.18 | 3.92 | 86 |
| Dakota | | 96 | 1.86 | 6.22 | 94 |
| | | 138 | 1.64 | 4.49 | 121 |
| | | 109 | 1.40 | 4.33 | 80 |
| | | 101 | 1.10 | 3.41 | 59 |
| | | 103 | 1.28 | 4.38 | 70 |
| Average | 43.5° F. | 115 | 1.42 | 4.93 | 86 |
| | | -- | 0.89 | 3.72 | 40 |
| | | 84 | 1.00 | 4.43 | 27 |
| Nebraska | | -- | 1.16 | 3.93 | 34 |
| | | 60 | 2.06 | 3.77 | 75 |
| | | 72 | 0.84 | 4.02 | 100 |
| | | 84 | 1.17 | 4.31 | 16 |
| | | 55 | 2.25 | 5.00 | 27 |
| | | 84 | 0.78 | 4.79 | 42 |
| | | 70 | 1.43 | 5.13 | 15 |
| | | 93 | 1.35 | 3.33 | 68 |
| | | 82 | 1.31 | 5.28 | 39 |
| | | 74 | 1.68 | 5.25 | 55 |
| | | 83 | 1.86 | 4.72 | 65 |
| | | 100 | 0.70 | 4.20 | 85 |
| | | 136 | 1.25 | 5.04 | 15 |
| | | 67 | 1.31 | 4.57 | 20 |
| | | 85 | 1.86 | 4.94 | 57 |
| | | 64 | 1.70 | 5.73 | 83 |
| | | 111 | 1.11 | 4.70 | 91 |
| | | 110 | 1.35 | 5.25 | 81 |
| | | 93 | 1.31 | 4.50 | 54 |
| | | 72 | 0.93 | 5.45 | 59 |
| | | 78 | 2.00 | 5.10 | 32 |
| | | -- | 1.62 | -- | 118 |
| | | -- | 1.47 | -- | 94 |
| | | -- | 2.18 | -- | 72 |
| | | -- | 1.92 | -- | 84 |
| | | -- | -- | -- | 85 |
| Average | 49.6° F. | 84 | 1.42 | 4.65 | 58 |
| | | 74 | 1.92 | 4.34 | 50 |
| | | 87 | 1.27 | 4.31 | 54 |
| Kansas | | 79 | 1.17 | 3.81 | 42 |
| | | 77 | 1.00 | 3.49 | 37 |

| State | Mean Annual Temperature | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|
| | | 96 | 0.90 | 4.03 | 55 |
| | | 100 | 1.08 | 4.62 | 83 |
| | | 79 | 1.56 | 4.26 | 46 |
| | | -- | 1.10 | -- | -- |
| Average | 55.5° F. | 84 | 1.25 | 4.12 | 52 |
| | | 108 | 1.10 | 4.13 | 69 |
| | | 78 | 0.92 | 3.70 | 38 |
| Oklahoma | | 66 | 0.48 | 1.88 | 15 |
| | | 67 | 0.65 | 3.06 | 22 |
| | | 65 | 1.04 | 4.82 | 36 |
| | | 60 | 0.60 | 1.55 | 20 |
| | | 37 | 0.21 | 1.08 | 4 |
| Average | 60.4° F. | 69 | 0.71 | 2.89 | 29 |
| | | 16 | 1.17 | 3.10 | 18 |
| | | 38 | 0.89 | 1.84 | 11 |
| Texas | | 37 | 0.68 | 3.61 | 18 |
| | | 63 | 0.36 | 3.15 | 12 |
| | | -- | -- | 3.21 | -- |
| | | -- | -- | 1.01 | -- |
| Average | 64.3° F. | 39 | 0.77 | 2.65 | 15 |

TABLE 2A

Along Western Isohyet Line

| State | Mean Annual Temperature | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|
| | 37.4 | 85 | 1.25 | 3.52 | 55 |
| North | 39.6 | 100 | 1.13 | 3.68 | 63 |
| Dakota | 40.0 | 118 | 1.22 | 4.21 | 81 |
| | 40.9 | --- | ---- | ---- | -- |
| Average | 39.5 | 101 | 1.20 | 3.80 | 66 |
| | 44.1 | 51 | 1.20 | ---- | 33 |
| Nebraska | 46.2 | 73 | 1.39 | 3.77 | 58 |
| | 47.9 | 95 | 0.72 | 3.00 | 64 |
| | 46.6 | 77 | 1.18 | 3.77 | 49 |
| | 46.6 | 44 | 0.73 | ---- | 18 |
| | 47.8 | 41 | 3.61 | ---- | 73 |
| | 48.3 | 77 | 1.07 | 4.21 | 43 |
| | 46.2 | 71 | 1.00 | 3.58 | 40 |
| | 48.5 | 77 | 1.02 | 3.67 | 41 |
| | 48.7 | 76 | 1.36 | 3.84 | 56 |
| | 49.5 | 94 | 1.52 | ---- | 76 |
| | 49.7 | 76 | 1.14 | 3.00 | 48 |
| | 51.0 | 85 | 1.20 | 3.72 | 54 |
| | ---- | 91 | 1.13 | ---- | 55 |
| Average | 48.25° F. | 74 | 1.31 | 3.61 | 50 |
| | | 70 | 0.90 | 2.77 | 36 |
| Colorado | 45.7 | 66 | 0.95 | 4.52 | 33 |
| | 48.4 | 72 | 1.13 | 3.72 | 41 |
| | 50.7 | 58 | 1.34 | 4.45 | 40 |
| | 50.4 | 65 | 0.99 | 3.78 | 35 |
| | 53.6 | 71 | 0.54 | 2.20 | 20 |
| | ---- | 75 | 0.66 | 2.46 | 27 |
| | ---- | 28 | 0.33 | 1.96 | 5 |
| | ---- | 61 | 0.48 | 1.87 | 16 |
| | ---- | 67 | 1.09 | 3.95 | 40 |
| Average | 49.7 | 64 | 0.84 | 3.17 | 27 |
| | 51.8 | 88 | 0.75 | 2.72 | 31 |
| Kansas | 53.5 | 96 | 0.63 | 1.61 | 32 |
| | 53.7 | 95 | 0.75 | 2.54 | 38 |
| | 54.4 | 93 | 0.64 | 2.28 | 32 |
| | 54.0 | 78 | 0.89 | 3.62 | 37 |
| | ---- | 71 | 0.77 | 3.21 | 30 |
| | ---- | 68 | 0.77 | 2.76 | 28 |
| | ---- | 103 | ---- | 3.00 | -- |
| | ---- | -- | ---- | 3.59 | -- |
| Average | 53.5 | 87 | 0.74 | 2.81 | 32 |

| State | Mean Annual Temperature | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|
| | 58.2 | 65 | 1.25 | 2.61 | 34 |
| Texas | 59.2 | 26 | 2.11 | 3.14 | 29 |
| | 59.5 | 7 | 4.2 | 2.09 | 16 |
| | 62.3 | 27 | 2.7 | 2.01 | 39 |
| | 65.1 | — | ---- | ---- | — |
| Average | 61. | 31 | 2.6 | 2.46 | 29 |

TABLE 3A

Samples Listed Along Isotherm Across
Nebraska and Colorado

| County | Mean Annual Precipitation (inches) | N.S.Q. | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|---|
| Douglas | 30-32 | 225 | 84 | 1.31 | 5.04 | 55 |
| Dodge | 28-30 | | 77 | 1.26 | 4.77 | 53 |
| Saunders | 28-30 | 205 | 70 | 1.68 | 4.89 | 66 |
| Lancaster | -- | | 74 | 1.31 | 3.99 | 57 |
| Butler | 28-30 | | 93 | 1.86 | 5.13 | 86 |
| Clay | 26 | | 100 | 1.70 | 4.72 | 91 |
| Hall | 24-26 | | 80 | 1.85 | 5.06 | 84 |
| Howard | 24-26 | | 60 | 0.97 | 4.22 | 51 |
| Kearney | 26-28 | | 81 | 1.40 | 4.47 | 61 |
| Average | 27.5" | | 77 | 1.54 | 4.70 | 64 |
| | | CENTRAL NEBRASKA | | | | |
| Sherman | 22-24 | | 46 | 0.97 | 3.87 | 24 |
| Custer | 22-24 | | 71 | 1.86 | 5.50 | 69 |
| Dawson | 20-22 | 143 | 48 | 0.65 | 2.00 | 17 |
| Frontier | 22+ | | 89 | 0.97 | -- | 46 |
| Hayes | 22+ | | 85 | 1.21 | -- | 53 |
| Average | 22 | | 68 | 1.13 | 3.79 | 42 |
| | | WESTERN NEBRASKA AND EASTERN COLORADO | | | | |
| Chase | 18-20 | | 76 | 1.16 | 3.00 | 48 |
| Perkins | 18-20 | | 94 | 1.53 | -- | 76 |
| Duel | 18 | | 76 | 1.35 | 3.84 | 56 |
| Cheyenne | 16-18 | | 72 | 1.12 | 3.67 | 43 |
| Kimball | 17 | | 71 | 1.01 | 3.58 | 40 |
| Banner | 16" | | 77 | 1.09 | 4.21 | 43 |
| Colorado | | | | | | |
| Logan | 17.6 | | 80 | 0.87 | 3.32 | 40 |
| Sedgwick | 17.4 | | 72 | 1.10 | 3.72 | 41 |
| Yuma | 17.4 | | 64 | 1.00 | 3.81 | 35 |
| Phillips | 17.6 | | 58 | 1.34 | 4.45 | 40 |
| Weld | 13.8 | 76 | 70 | 0.94 | 2.77 | 35 |
| Average | 16.7 | | 73 | 1.13 | 3.63 | 45 |
| | | WYOMING | | | | |
| Goshen | 14" | | 46 | 1.06 | 3.66 | 30 |

TABLE 4A

Samples Listed Along Isotherm Across

Kansas and Colorado

| County | Mean Annual Precipitation (inches) | Relative Humus Color | Relative Per cent Humus HC=10 | Per cent Organic Matter HC=10 | Relative Pigment Content HC=10 |
|---|---|---|---|---|---|
| | | KANSAS | | | |
| Marion | 30.35 | 80 | 1.27 | 3.98 | 49 |
| Butler | --- | 79 | 1.10 | 4.26 | 47 |
| McPherson | --- | 100 | 1.56 | 4.62 | 82 |
| Sedgwick | 30.14 | 78 | 1.85 | -- | 77 |
| Average | 30.2" | 84 | 1.44 | 4.28 | 73 |
| | | WESTERN KANSAS | | | |
| Ellis | --- | 71 | 0.77 | 3.22 | 30 |
| Trego | --- | 78 | 0.90 | 3.59 | 37 |
| Scott | 19.93 | 90 | 0.64 | 2.28 | 32 |
| Ford | 20.4 | -- | 0.95 | 3.15 | -- |
| Finney | 19.5" | 68 | 0.77 | 2.76 | 28 |
| Average | 19.9" | 77 | 0.80 | 2.98 | 32 |
| | | COLORADO | | | |
| Prowers | 15" | 75 | 0.66 | 2.46 | 28 |
| Otero | -- | 28 | 0.33 | 1.96 | 5 |
| Las Animas | 12.5 | 61 | 0.48 | 1.87 | 15 |
| Average | 13.7" | 55 | 0.49 | 2.09 | 16 |